# TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE

**A THESIS SUBMITTED TO THE BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT (PUNJAB)**

**For the Award of Degree of**

**DOCTOR OF MEDICINE**

**IN**

**MEDICINE**

**BY**

**Dr. UDAY PRATAP SINGH**

**SUPERVISOR**

**Dr. DINESH JAIN**

**DEPARTMENT OF MEDICINE**

**DAYANAND MEDICAL COLLEGE AND HOSPITAL**

**LUDHIANA, PUNJAB**

**(MAY 2021)**

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I declare that the thesis entitled **"TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE"** has been prepared by me under the guidance of **Dr. Dinesh Jain**, Professor, Department of Medicine Dayanand Medical College and Hospital, Ludhiana. No part of this thesis has formed the basis for the award of any degree previously.

_____

**Dr. Uday Pratap Singh**

Department of Medicine

Dayanand Medical College and Hospital

Ludhiana, Punjab

# CERTIFICATE

I certify that **Dr. Uday Pratap Singh** has prepared the thesis entitled **"TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE"** for the award of **M.D. Degree** of Baba Farid University of Health Sciences, Faridkot. He has carried out the work at the Department of Medicine, Dayanand Medical College & Hospital, Ludhiana

The data collected in this thesis is genuine and the work has been carried out by the candidate himself.

**Supervisor**

**Dr. Dinesh Jain**
Professor,
Department of Medicine,
DMC&H, Ludhiana

**Co-Supervisor**

**Dr. Varun Mehta,**
Professor,
Deptt of Gastroenterology,
DMC&H, Ludhiana

Date:

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**Head of Department**

**Dr. Dinesh Gupta**
Professor & Head
Department of Medicine
DMC&H, Ludhiana

**Principal**

**Dr. Sandeep Puri**
Principal
DMC&H, Ludhiana

Date:

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**Dr. Uday Pratap Singh**

Department of Medicine

Dayanand Medical College and Hospital,

Ludhiana, Punjab.

Date:

# ACKNOWLEDGEMENTS

No hard task is complete without taking small but certain steps. And no matter what accomplishments you achieve, somebody always helps you. The difficulties in performing any task start disappearing if pursued with honesty, perseverance and support from people around who are always ready to help. But in actuality, this thesis is a product of efforts of many individuals and the very least I can do to be thankful to them is to acknowledge their support. I am grateful to **GOD ALMIGHTY** with whose blessings this thesis project could be completed. I am sincerely grateful to many generous people, for sharing their vast knowledge, truthful and illuminating views on a number of issues related to this research.

First and foremost, a definite honorable mention goes for my father, **Dr. Satendra Kumar Singh**, my mother, **Mrs. Anuradha Singh** and my brother **Dr. Dhruv Pratap Singh** ,without whose love and blessings nothing would have been possible. It is their guiding light which has always helped me fight in the toughest of times. It would only be appropriate to begin my note of gratitude by thanking them.

I feel indebted for life to my learned guide, revered teacher and illustrious supervisor, **Dr Dinesh Jain, Professor,** Department of Medicine, Dayanand Medical College and Hospital, Ludhiana. His knowledge and dedication not only made me believe in the science of

*Internal Medicine, but also made me work hard with zeal towards my subject. His depth of knowledge, readiness to help, understanding attitude, concern for excellence, limitless patience and able supervision have made this thesis a reality and also made me a wiser, humble and dedicated physician.*

*I am blessed to have* **Dr. Varun Mehta, Professor,** *Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, as my thesis co-supervisor. His vast experience, in-depth analysis of the subject and attention of detail have been a big helping hand in my thesis. His constant motivation, out of the box thinking and dedication have instilled in me discipline and sincerity for my subject and patients.*

*I bow down with gratitude to all my teachers, specially to* **Dr. Dinesh Gupta, Professor & Head,** *Department of Medicine, Dayanand Medical College and Hospital, Ludhiana and* **Dr. Sandeep Puri, Professor** *of Medicine &* **Principal,** *Dayanand Medical College and Hospital, Ludhiana for their constant motivation. They always kept me on the right path, pushing my limits to excel in every aspect.*

*I also extend my gratitude to all my consultatnts* **Dr. Rajesh Mahajan, Dr. Vandana Midha, Dr. Gautam Ahluwalia, Dr. N.P. Jain, Dr. H.S. Dhooria, Dr. Vikas Garg, Dr. Amit Berry, Dr. Sandeep Chhabra, Dr. Vipin Kumar** *and* **Dr. Jyoti Jindal** *for their invaluable guidance.*

*I wish to place my deep sense of gratitude, gratefulness and sincere regards to,* **Dr. Parth Bansal, Dr. Sandeep kaur, Dr. Anterpreet , Dr. Meenal, Dr. Anurag, Dr. Tanveer** *and* **Dr. Sahil,** *who as seniors helped and guided me during the course of this study.*

*My thesis would be incomplete without the help of* **Dr. Arpit, Dr. Ishaan, Dr. Rajneesh, Dr. Kapil, Dr. Aanchal, Dr. Navjot, Dr.Twinkle, Dr. Tanya and Dr. Anureet** *who constantly motivated me in my thesis study.*

*A special thanks to* **Dr. Tanveer,** *Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, who went out of his way for helping me in my thesis.*

*My sincere thanks to* **Dr Sanjana Jain** *for the motivation & support and for always providing a helping hand whenever needed.*

*Lastly, my note of gratitude would be incomplete without thanking my patients and their families for their co-operation and patience for the entire course of my study. They are the backbone of this study, and deserved a special mention.*

*Dr. Uday Pratap Singh*

# ABSTRACT

**Background**: The prevalence of NAFLD has greatly increased in patients suffering from pre-existing manifestations of the metabolic syndrome, such as obesity, type 2 diabetes, hyperlipidemia and hypertension. The main objectives of this study were to find a correlation between degree of fibrosis and serum ferritin levels in patients of NAFLD and to define values of serum ferritin which could differentiate high and low grade of fibrosis.

**Material and Methods:** A prospective observational study was conducted over 100 patients in the Department of Medicine and Gastroenterology, Dayanand Medical College and Hospital, Ludhiana over a period of one year. After Ethical Committee clearance, patients diagnosed with NAFLD were selected after considering specific inclusion and exclusion criteria. A detailed proforma including detailed history, examination and the laboratory data of the patient was recorded for each patient. Patients were evaluated for metabolic syndrome components like obesity, diabetes, hypertension and hypertriglyceridemia. Levels of serum ferritin were sent for all patients and degree of fibrosis was noted on fibroscan.

**Results:** In a total of 100 patients with NAFLD included in the study, the mean age was 45.99 +- 12.17 years with a male predominance. It was seen that 52% had obesity, 71% were diabetic and 80% had hypertriglyceridemia. A statistically significant correlation was seen between hyper ferritinemia and underlying degree of liver fibrosis( p-value 0.001). Majority of the patients with serum ferritin > 500ng/dl had a higher grade of fibrosis.

**Conclusion:** We concluded that grades of fibrosis in NAFLD was higher in patients of advancing age, presence of metabolic syndrome components i.e. obesity, diabetes mellitus and hypertriglyceridemia with a male predilection. There is a significant relationship between serum ferritin levels and liver stiffness as seen on fibroscan in patients with NAFLD. Thus, we can use serum ferritin level as a noninvasive, easy available and an economic option for predicting the severity of liver fibrosis among NAFLD patients.



**Key words:** NAFLD, Ferritin levels, Fibroscan, metabolic syndrome

# ABBREAVIATIONS

| | | |
|---|---|---|
| 1 | BMI | Body Mass Index |
| 2 | DM | Diabetes Mellitus |
| 3 | FL | Fatty liver |
| 4 | HFE | High iron gene |
| 5 | HTN | Hypertension |
| 6 | LSM | Liver stiffness measurement |
| 7 | NAFLD | Non alcoholic fatty liver disease |
| 8 | NASH | Non-alcoholic steatohepatitis |
| 9 | SF | Serum ferritin |
| 10 | SGOT/AST | Serum Glutamic-oxalacetic transaminase |
| 11 | SGPT/ALT | Serum Glutamic-pyruvic transaminase |

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# INTRODUCTION

NAFLD has become the leading cause of chronic liver disease and affects 30-40% of the world population. The prevalence of NAFLD has greatly increased in patients suffering from pre-existing manifestations of the metabolic syndrome, such as obesity, type 2 diabetes, hyperlipidemia and hypertension. NAFLD is prevalent in 70% of the diabetics and in 90% of the morbidly obese essentially making it the hepatic component of the metabolic syndrome.

NAFLD is non alcoholic fatty liver disease which refers to presence of hepatic steatosis when no other causes for secondary fat accumulations are present.

Based on the presence or absence of hepatocyte injury, inflammation and histological appearance, NAFLD can further be classified into NAFL (non-alcoholic fatty liver) and NASH (non- alcoholic steatohepatitis). NAFL is a milder, asymptomatic and a more easily reversible condition with hepatic steatosis without evidence of significant inflammation. On the other hand, NASH is a more severe stage of the disease with inflammation and ballooning degeneration of hepatocytes and can result in chronic liver damage, scarring and fibrosis. The progression of NAFL to NASH occurs in 20-30% of the patients, but the conditions remain clinically indistinguishable. NASH leads to continuous liver fibrosis and may develop in to cirrhosis in 10-15% of the patients over a period of 10 years, in which hepatocyte loss occurs causing a functional impairment.

NAFLD has been recognized as the major risk factor in the etiology of HCC in developed and developing nations.

NAFLD has a pathophysiological relationship with hyperinsulinemia, proatherogenic state, and changes in iron metabolism evidenced by hyperferritinemia (HPF) and hemochromatosis, which may have multiple meanings in these patients, since iron accumulation triggers formation of free radicals and damage to cellular function. Hyperferritinemia is a frequent finding detected in 30%–40% of the patients with MS/NAFLD,

and has been suggested as a marker of severity of the disease. Relationship between hyperferritinemia and IR seems to be mutual. Insulin determines a rapid and marked stimulation of iron uptake by fat cells, by a redistribution of transferrin receptors from an intracellular membrane compartment to the cell surface. Adiponectin, which is an anti-steatotic and anti-inflammatory adipokine, is reduced in dysmetabolic conditions like NAFLD, IR and T2DM, and seems to predict the severity of liver inflammation and fibrosis.

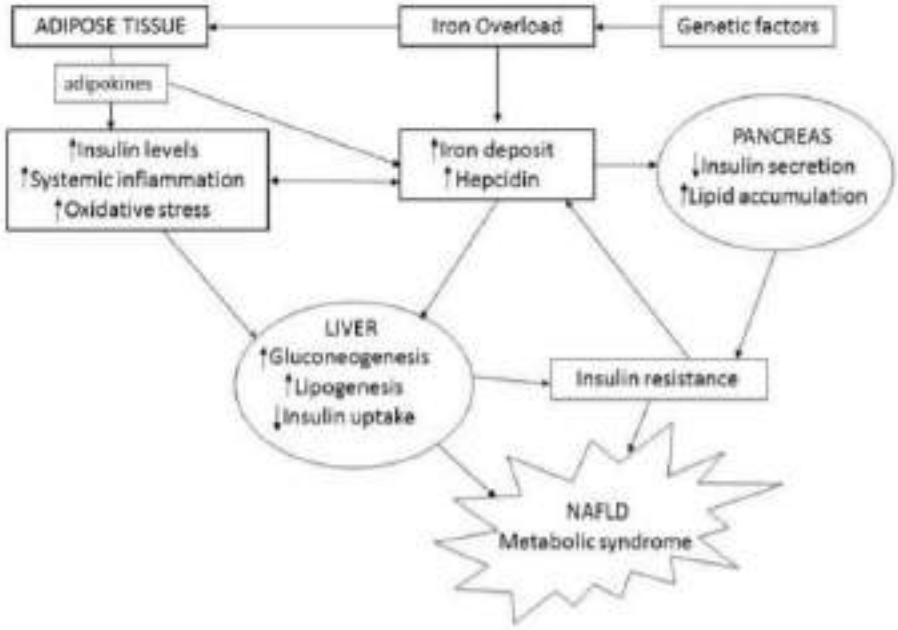
The diagnosis is made by:

1. Demonstration of hepatic steatosis by imaging or biopsy
2. Exclusion of significant alcohol consumption
3. Exclusion of other causes of hepatic steatosis.

Liver biopsy is the gold standard in diagnosing NAFLD but it has its own limitations i.e. being an invasive method, complications like pain & hypotension, length of hospital stay & cost sampling variations & low level of patients satisfaction. Recently various non invasive modalities have been introduced to estimate the degree of fibrosis and one of them being transient elastography. Fibroscan uses ultrasound for determining the liver stiffness and degree of fibrosis. Serial evaluation of liver stiffness can provide evidence of progression of NAFLD.

Several biomarkers have also been reported for prediciting the severity of disease. NAFLD Fibrosis Score is a widely validated scoring system which is based on six variables (age, BMI, hyperglycemia, platelet count, albumin, ALT/AST ratio). Serum ferritin is associated with severity of hepatocellular aggression. Ferrtin being an acute phase reactant is found to be raised in several systemic inflammations such as rheumatological, infectious and neoplastic diseases. Hyperferritinemia has been detected in about one-third of the patients with NAFLD and Metabolic syndromes. Elevated serum iron and ferritin has been found in patients of NAFLD without underlying genetic hemochromatosis or iron accumulation in the liver. It directly correlates with the severity of fibrosis. Firstly, iron

accumulated in liver causes oxidative stress with ROS production and damage to membranes, proteins and DNA. Secondly ferritin being a pro-inflammatory cytokine activates hepatic stellate cells via NFKB cascade and induce fibrogenesis.




Hepcidin plays a key role in iron accumulation in NAFLD & increased levels of this peptide has been found in these patients. Hyperferrintemia has also been implicated in the development of insulin resistance by impairing the pancreatic beta cell function due to intra-parenchymal iron deposition. Insulin stimulates ferritin synthesis via inflammatory pathways and enhances hepcidin expression.

NAFLD is recognized as the leading cause of chronic liver disease worldwide, and, in a percentage of cases, it is potentially progressive towards advanced fibrosis and severe complications. As a consequence, the need for predictive factors of NAFLD and especially of its progressive forms is mandatory. Thus, this study was conducted to find the correlation of ferritin levels and Fibroscan with the severity of the disease.

# AIMS AND OBJECTIVES

1. To find a correlation between degree of fibrosis and serum ferritin levels in patients of NAFLD.
2. To define values of serum ferritin which could differentiate high and low grade of fibrosis.

# REVIEW OF LITERATURE

Ford et al.[1] reported the results of a case-control study in Europe, demonstrating that subjects with hyperferritinemia had a 2.4-fold higher risk of developing T2DM.

Salonen et al.[2]conducted a study to demonstrate correlation between S.ferritin levels and liver involvement and concluded increased ferritin levels precede the development of DM.

Moirand R et al coined the term “dysmetabolic iron overload syndrome” (DIOS), to indicate subjects with increased ferritin levels, with normal or only mildly increased transferrin saturation, in the presence of liver steatosis, IR and two or more components of the MS, along with moderate hepatic iron accumulation with the typical pattern of mixed parenchymal and mesenchymal iron deposition. It was observed that several patients with NAFLD, IR and manifestations of MS may have increased ferritin even in the absence of increased iron stores.[3]

S.Fargion et al conducted a study to demonstrate correlation between S.ferritin levels and liver involvement and concluded that hyperferritinemia with normal transferrin saturation was a hallmark of a glucose/lipid metabolism disorder and, when associated with multiple metabolic abnormalities and iron overload, identified patients at risk for NASH. It was observed that patients in whom ferritin remained elevated despite lifestyle modifications (diet, weight loss, physical activity) differed from those whose ferritin normalized, presenting the former a more severe liver disease.[4]

Manousou et al.[5], conducted a study in 2010-11 at Royal Free Hospital, London to evaluate the role of serum ferritin as a discriminant marker for both inflammation and fibrosis in NAFLD patients diagnosed histologically. 111 patients were included in the study with a median age of 52.6 years, 64 males and 62 obese patients. 58 patients had diabetes mellitus, 26 had hypertension and 40% had hyperlipdemia. Results showed that on histology, 40.7% had fatty liver, 30.6% had borderline NAFLD and 28.7%

had NAFLD whereas 11% had liver cirrhosis. It was concluded that serum ferritin levels and BMI have a strong association with fibrosis, portal and lobular inflammation in these patients and hence are discriminant markers to take patients for liver biopsy and are associated with inflammation and fibrosis.

Kowdley et al.[6] demonstrated that elevated serum ferritin is an independent predictor of histologic severity and advanced fibrosis among patients with NAFLD. He found in a cohort of 628 biopsy-proven NAFLD with hyperferritinemia that ferritin, besides being significantly associated with markers of liver damage (elevated serum ALT, AST and decreased platelets) and of iron overload (iron, transferrin-iron saturation and iron stain grade), was associated with more severe histologic features of NAFLD, including steatosis, hepatocellular ballooning, increased NAFLD Activity Score (NAS) and diagnosis of NASH.

A total of 628 NASH adult subjects (age≥18 years) with biopsy proven NAFLD (defined as >5% steatosis) and a serum ferritin measurement within six months of their fibroscan were evaluated. Subjects were divided according to sex and threshold SF value of 1.5XULN (i.e., >300 ng/ml in women and >450 ng/ml in men). Higher proportion of patients with SF>1.5XULN showed increased severity of steatosis, lobular inflammation. and fibrosis compared to patients with SF≤1.5XULN (p≤0.026). Subjects with SF >1.5XULN were also more likely to have a definite diagnosis of NASH than those with SF ≤1.5XULN (70% vs. 59%, p=0.017). The proportion of subjects with advanced (stage 3 or 4) fibrosis at higher SF levels was 32% of SF>1.5XULN, 42% SF>2.5XULN and 34% SF>3.5XULN. To investigate whether various threshold levels of elevated SF were independently associated with advanced fibrosis, a multivariate stepwise logistic regression analysis including the following variables selected a priori, known to be associated with severity of NAFLD: age at, sex, BMI, presence of diabetes and ALT.

The study showed that serum ferritin has value as a non-invasive marker in identifying patients with NAFLD who are at increased risk of more advanced disease as indicated by a higher NAS, even among patients without hepatic iron deposition. It was found that SF>1.5XULN is independently associated with increased risk of advanced fibrosis in NAFLD patients. Results indicated that use of a SF value >1.5XULN can be of diagnostic value in patients with NAFLD.

Zelber-Sagi et al.[7] showed that NAFLD was responsible for the association between serum ferritin and the metabolic syndrome and most of its components suggesting that the relation between serum ferritin and most of metabolic syndrome features might be mediated by the presence of NAFLD at population-based level. Among the metabolic factors, insulin appeared to be the strongest predictor of increased serum ferritin levels, but the association between serum ferritin and insulin was much more evident in the NAFLD group.

Angulo et al.[8] retrospectively analyzed in 1404 NAFLD patients the accuracy of serum ferritin in determining the presence and severity of liver fibrosis, and whether combining non-invasive fibrosis scoring systems with serum ferritin analysis could increase the accuracy of those tests

Yoneda et al.[9], analyzed 1201 biopsy-proven NAFLD patients previously enrolled into the Japan Study Group of NAFLD and belonging to a large Japanese cohort database of NAFLD patients. Serum ferritin levels and hepatic histology were compared, the authors showed that ferritin increased with increasing histological grade of steatosis, lobular inflammation.

Bugianesi et al.[10] conducted a study and demonstrated that increased ferritin levels are markers of severe liver damage, but not of iron overload .The iron burden and HFE mutations do not contribute significantly to hepatic fibrosis in the majority of patients with NAFLD was also proven.

Vecchi et al. [11],conducted a study and demonstrated the relationship between glucose and iron metabolism and showed a new regulatory

pathway in iron homeostasis which was driven by gluconeogenic stimuli with major actors being hepcdin and PPARGC1A, a transcriptional coactivator of genes involved in gluconeogenesis.

Dongiovanni et al. [12], conducted a study in which an iron-enriched diet led to the development of IR, due to the secretion of adipokines by the visceral adipose tissue consequent to iron accumulation

Kim C.W et al [13] conducted a study Serum ferritin levels predict incident non-alcoholic fatty liver disease in healthy Korean men and concluded that increased ferritin levels plays a role in development of DM.

Beckry et al. [14] showed the ectopic expression of hepcidin in white adipose tissue of obese individuals and leptin, which usually increased in obese subjects, was in turn was able to enhance hepcidin mRNA *in vitro.*

Valenti L, Fracanzani AL et al[15], carried out a randomized trial of iron depletion in patients suffering from NAFLD and hyperferritinemia in 2013. Subjects between the age of 18-75 years were enrolled in the study who underwent liver biopsy for ultrasonographically detected liver steatosis and hyperferritinemia, ferritin levels >250ng/ml and NAFLD score >1. Iron depletion was achieved by taking out 350ml of blood every 10 to 15 minutes until ferritin <30ng/ml and transferrin saturation < 25%. Total of 38 patients were randomized 1:1 to phlebotomy (n=21) or lifestyle changes (n=17). The main outcome was the improving liver damage according to the score at 2 years. Phlebotomy was associated with normalization of iron parameters. A better improvement was seen in steatosis grade in iron depleted as compared to the control patients (p value = 0.02). Patients were followed up after 2 years, a decrease was observed in the levels of ALT, AST and GGT (P<0.005). The effect of iron depletion on liver damage improvement as assessed by histology or ALT decrease >20% was independent of baseline AST/ALT ratio and insulin resistance (P value = 0.0001).

Dongiovanni P [16] showed that HFE mutations responsible for hereditary hemochromatosis resulted non significantly associated either to liver

siderosis or hyperferritinemia and also liver damage did not result as being influenced by the presence of these mutations.

Facchini *et al* [16] demonstrated that NAFLD patients, with or without increased ferritin levels, that iron removal in patients with clinical evidence of nonalcoholic fatty liver disease was able to improve insulin sensitivity in the short term

Alam *et al.* [17] demonstrated that obesity led to hyperferritinemia irrespective of actual body iron story, advocating a state of subclinical inflammation responsible for high levels of ferritin and increased BMI leads to hyperferritinemia.

Iwasaki T et al[18] conducted a study showing an association between serum ferritin, visceral fat and subcutaneous adiposity and thereby making serum ferritin concentration a useful indicator of systemic fat content and degree of IR

Green et al [19] demonstrated that isolated adipocytes exposed to iron become insulin-resistant decreasing insulin mediated glucose transport and fostering lipolisis.

Nakamura A, Yoneda M et al[20] reinvestigated the clinical usefulness of the modified NAFIC scoring system in the Japanese patients suffering from NAFLD and had undergone liver biopsy. A total of 147 patients with the disease were included in the study. 355 patients from various hepatology centres were also enrolled for validation. In this study, it was seen that 74 (50.3%) patients were histologically diagnosed with NASH, whereas the rest 73 (49.7%) were found without NASH. Percentage of patients with NASH was seen with higher insulin levels about > or = 15 microU/mL. Thus the score was modified to the use of 1 point for insulin greater than 10 and 2 points for serum levels greater than or equal to 15microunits/ml. It showed improved sensitivity and the findings were also confirmed in the patients included in the validation group. At the end, they also concluded that this scoring could be used as a clinically useful diagnostic screening tool for NASH.

George et al[21], conducted a study to investigate the role of the hemochromatosis mutation Cys282Tyr in the development of the mild hepatic iron overload in NASH patients and its correlation with hepatic damage. 51 patients in total were enrolled in the study and all were tested for the chromosome. This data was analysed according to the histological grade of steatosis, inflammation, Perls' staining, hepatic iron concentration (HIC), and serum iron indices.

31% patients with NASH were either homozygous or heterozygous with the mutation chromosome. The mutation was significantly associated with Perl's stain grade (p value < 0.005), HIC (p value < 0.005) and transferrin saturation percentages (p value < 0.005) but not with serum ferritin levels. Thus, it was concluded that Cys282Tyr gene mutation is responsible for most of the mild iron overload found in the NASH and has an impact on the hepatic damage.

Bugianesi E. et al[22] conducted a study in 2002 to define a relative contribution of iron burden, HFE mutations and insulin resistance on the severity of liver fibrosis in NAFLD. In this cohort study, 263 patients were included. Out of the total, 7.4% patients showed signs of peripheral iron overload whereas 9% had hepatic iron overload, but 21.1% had hyperferritinemia. The prevelance of C282Y and H63D HFE mutations was similar to the other general population and had no association with iron overload. It was seen that the presence of severe fibrosis was associated independently with older age, females, overweight, aspartate/alanine aminotransferase ratio, serum ferritin level, fasting glucose level and insulin levels, decreased insulin sensitivity and with histologic features (degree of necroinflammation and steatosis). After altering the BMI of the patients, it was seen that age, sex and degree of steatosis, ferritin levels and oral glucose insulin sensitivity were independent predictors of severe fibrosis. Thus, he concluded after the study that insulin resistance is a main, independent risk factor for advanced fibrosis in patients with NAFLD. Increased ferritin levels are

markers of severe histologic damage but not of iron overload. It was also mentioned that there was no contribution of iron burden and HFE mutations in the hepatic fibrosis in major percentage of NAFLD patients.

Chen S, Huang Y F et al[23], investigated the association of hyperferritinemia and hyperuricemia with liver function abnormality in obese adolescents. The survey included 2090 adolescents from different high schools. Anthropometry and biochemical measurements along with the basic laboratory investigations were perfumed on all students. An ALT>42 U/L was defined as elevated, a ferritin level >200 µg/L was defined as hyperferritinemia. A uric acid level >460 µmol/L in males and >340 µmol/L in females was defined as hyperuricemia. The data was analyzed using chi-sqaure test, linear regression and multivariate logistic regression. 76 patients (3.6%) were found with elevated ALT level and was more prevalent in males than females (p value < 0.001). The logistic regression analysis found that body mass index, hyperferritinemia and hyperuricemia were significant factors associated with the ALT elevation, but gender, age, and white blood cell count were not. Thus, it was found that hyperferritinemia and hyperurecemia are two significant factors in relation to the elevated ALT levels among the obese adolescents.

Kim Chan-Won et al[24], aimed to examine whether serum ferritin levels can predict incident fatty liver in non-diabetic men. The cohort study comprised of 2410 healthy male aged between 30 to 59 years of age with no evidence of ultrasonographically detectable fatty liver. Every visit of the patient included biochemical and anthropometric measurements and abdominal ultrasound. The hazard ratio (95% confidence interval) for incident USFL comparing the highest quartile of serum ferritin level to the lowest quartile was 1.54 (1.21–1.94) after adjusting for age, body mass index, smoking, alcohol intake, and exercise. Thus in the end it was found that increased serum ferritin levels appear to be an early predictor for incident fatty liver.

Yoneda M, Thomas E et al[25], conducted a study in 2014 to see the clinical usage of serum ferritin to assess liver fibrosis in patients with non-alcoholic fatty liver disease. A total of 1201 biopsy proven NAFLD patients were enrolled in the study. Serum ferritin levels were compared to the hepatic histology. It was found that serum ferritin increased with the increasing histological grade of steatosis, lobular inflammation and ballooning. Due to correlation of ferritin levels with various factors like sex differences, steatosis and fibrosis, it was found to have low diagnostic accuracy for specifically detecting liver fibrosis in NAFLD patients due to involvement of various hepatocellular processes.

Kim Y S, Jung E S et al[26], conducted a study to find non invasive predictors of non alcoholic steatohepatitis in patients with histologically proven NAFLD. The aim of the study was to identify some clinically useful non invasive parameters to distinguish between NASH and NAFLD and also to determine the correlation of levels of identified parameters with the severity of liver injury in patients with NASH. 108 biopsy proven patients with NAFLD were enrolled in the study. 67 patients (62%) had NASH. Out of the patients having subtype 3 and 4 of NAFLD showed tge NAFLD histologic score of greater than 3 points. 40.3% had a score of 3 or 4 points whereas 59.7% had >4 points score. Fragmented CK 18 showed association with NAS and various components of NAS like ballooning, lobular inflammation and also with various laboratory markers like asparate aminotransferase, alanine aminotransferase, serum ferritin and the fibrosis stage. Thus, at the end of the study, it was concluded that serum fragmented CK 18 helps in distinguishing between NASH and NAFLD whereas combined with serum ferritin, helps in improving the diagnostic performance of this division.

Kalambokis G et al[27], carried out a study in 2011 at London to determine serum ferritin as a discriminant marker for both fibrosis and inflammation in NAFLD patients who were proven histologically. Serum ferritin and features of metabolic syndrome in relation to histological inflammation or

fibrosis were evaluated in NAFLD patients. A total of 111 patients were included in the study with a median age of 52.6 years. Out of the total number of patients, 40.7% had fatty liver, 30.6% had borderline NASH, 28.7% had NASH and the rest 11% had cirrhosis. Multivariate regression indicated that serum ferritin, diabetes, BMI and AST were independently associated with NASH. They together covered an area of 0.91 in the ROC curve. Therefore, they concluded at the end that serum ferritin and BMI have a strong association with fibrosis, portal and lobular inflammation in NAFLD patients. They also stated that both can be used as a potential discriminant markers to select patients to undergo liver biopsy and are associated with inflammation and fibrosis.

Seyedian SS, Hajiani E et al[28]conducted a study to determine the relationship between serum ferritin levels and transient elastography findings in the patients with NAFLD. In this study, all the patients attending hepatology clinic during a 4-months period were included and the ones <18 years of age, with history of alcohol consumption (>20g/d) , ALT > 150, history of any advanced chronic liver disease, parentral nutrition in last 6 months, history of GI surgery, heart failure, any other liver pathology like positive viral markers, Wilson's disease etc. were excluded.

All the participants enrolled in the study were evaluated for obesity, hypertension, uncontrolled diabetes and hyperlipidemia. Later, all patients underwent liver elastography to determine the liver stiffness based on KPa and serum ferritin was sent. The mean serum ferritin level among men with mild and advanced liver stiffness was 154 ± 97 and 244 ± 214 respectively with a p value <0.001. These parameters in female patients had different values with mild and advanced liver stiffness 79 ± 91 and 161 ± 103 respectively with a p value = 0.003. The cutoff values for ruling out severe advanced liver stiffness in both the gender with 90% accuracy were 72.5ng/ml and 65.5ng/ml respectively. Thus, it was stated that there is a significant relationship between serum ferritin level and liver stiffness

among NAFLD patients and serum ferritin can therefore be used as a predictor of advanced liver fibrosis.

Moghaddasifar I et al[29] evaluated the prevalence of NAFLD and various related factors affecting the incidence of the disease. 23 studies including 25,865 individuals were entered in the study. They observed that NAFLD was more common in the male gender and higher age group. Various factors like DM, other metabolic syndrome components like hypertension and hypertriglyceridemia affected the incidence of the disease.

Valenti L, Fracanzani AL et al[30] conducted a study in 2009 stuyding the characteristics of HFE genotype, parenchymal iron accumulation and liver fibrosis in patients with NAFLD. 587 patients with NAFLD were included in the study and 184 were taken as controls. It was seen that iron accumulation in hepatocytes was associated with a 1.7 fold higher risk of a fibrosis stage >1, compared with the absence of siderosis. Hepatocellular siderosis was seen to be more prevelant in patients with HFE mutations than in without them. At the end, it was concluded that the iron deposition mainly in the hepatocytes is associated with more severe liver damage in patients with NAFLD however, it cannot be used to identify patients with hepatocellular iron accumulation.

Chandok N, Minuk G et al[31], conducted a study to determine if serum ferritin levels predicted the underlying stage of NAFLD. In this study, the clinical, biochemical, radiologic and histologic findings of NAFLD adults were evaluated. 482 patients fulfilling the inclusion criteria were enrolled including 60 subjects with simple steatosis proven by biopsy, 28 subjects with steatohepatitis and 20 subjects with histologic or radiologic evidence of cirrhosis. Mean ferritin levels were seen to be similar in all the three groups. Univariate and multivariate analysis was done, it was further observed that only AST/ALT ratio, diabetes, splenomegaly and age accurately predicted the stage of underlying NAFLD induced liver injury. Thus, it was concluded that hyperferritinemia is common in patients with

NAFLD but the measure of the level elevation doesn't predict the stage of the disease existing.

Saadeh S et al[32], conducted a prospective study to establish the role of radiological modalities in diagnosis of NASH. Consecutive patients with biopsy proven NAFLD between 2000-2001 were included in the study excluding the ones having a significant alcohol consumption history. Each biopsy specimen taken was assessed and the patients also underwent limited abdominal ultrasound, computed tomography, and magnetic resonance imaging (MRI) and was interpreted by a team of radiologists independently. Patients with NASH had > AST levels (P value = 0.03), greater ferritin levels (P =0.05), more hepatocyte ballooning (P value < 0.0001) and more fibrosis (P value = 0.002). There was no difference seen in the radiological featured of NASH and NAFLD. The radiological pictures only showed featured important for making a diagnosis of NASH and its severity.

Angulo P et al[33] conducted a study check the accuracy of serum ferritin levels as a diagnostic parameter for liver fibrosis in patients of NAFLD. A retrospective study was conducted in 1014 patients with liver biopsy confirmed NAFLD. A greater proportion of patients with increased levels of serum ferritin had NASH and more advanced form of fibrosis than patients with no increased levels. It was however seen that there was no change in the score of the NAFLD with the addition of serum ferritin levels.

Prashanth M, Ganesh HK et al[34] conducted a study in 2009 over a period of two years and studied the prevalence and risk factors of NAFLD in patients with type 2 diabetes. 204 Type2 DM were included and underwent ultrasound abdomen. Out of these, 90 patients diagnosed with fatty infiltration gave a consent for liver biopsy and clinical as well laboratory workup. 87% of the patients were diagnosed with NAFLD on basis of histological findings with 37.3% fibrosis and the rest with steatohepatitis. At the end of the study, it was concluded that NAFLD had no effect on prevalence and severity with age, duration of diabetes,

glycemic control index, BMI and family history whereas it was seen that patients with steatohepatitis showed increased ALT levels. They concluded at the end that DM Type 2 patients are at a higher risk for NAFLD and NASH and the chances increase with the presence of multiple components of metabolic syndrome.

Golabi P, MD, et al. [35] carried out a study to assess the relationship of NAFLD and metabolic syndromes with long term outcome of mortality. Patients diagnosed with NAFLD with a median age of 43 years were included in the study with 73% whites and 50% males. NAFLD group with no syndrome was compared to NAFLD group with atleast one metabolic syndrome and was seen to have a significantly older, higher BMI, likely insulin resistance and a heart disease. Patients were followed up for over 19 years and in this period a total of 1039 patients expired. Hence, it was observed that NAFLD patients with an associated metabolic syndrome had a higher risk of mortality at 8 years and 16 years as compared to patients with no syndromes and therefore, should be treated on an early priority.

Vernon G et al(36) did a systematic review on the epidemiology of NAFLD and NASH in the adults. It was seen that the main diagnostic tools for NAFLD is liver biopsy, ultrasound and various blood investigations. They stated that NAFLD was common in patients with comorbid conditions like diabetes and obesity.

Chandok N, Minuk G et al[37], carried out a study in Canada in 2011 to determine ferritin levels as a predictor of severity of NAFLD. It was done over a period of 15 years and included a total of 482 patients with 60 patients with biopsy proven steatosis, 28 patients with NASH and 20 subjects with histology derived diagnosis. All three groups had the same mean ferritin values. They concluded in the end that though serum ferritin levels get elevated during the course of the disease but the rise in ferritin does not predict the severity or stage of the disease.

Canbakan B, Senturk H et al[38], in 2007 carried out a study to see the correlation of clinical, laboratory and histological factors in non-alcoholic patients with NAFLD. 105 non alcoholic patients with NAFLD were included in the study. Diabetic patients were seen to have more severe form of necroinflammation and fibrosis. Serum ferritin levels and ultrasonographic grade were seen to be higher in patients with NASH but showed low liver spleen density gradient. Thus, they concluded that NAFLD with elevated ALT have more severe form of liver disease. Ferritin levels have significant role in prognosis of liver damage and fibrosis.

Hagstrom H, Nasr P et al[39], carried out a study in 2016 and evaluated the association of elevated serum ferritin levels with probability of mortality in patients with NAFLD. Patients were followed up for 16 years. 222 patients meeting the inclusion criteria were enrolled in the study. At the end of the study, they concluded that patients having higher values of serum ferritin had more advanced form of fibrosis. However, there was no difference in the number of deaths amongst patients with different iron overload patterns.

Galarregui C, Cantero I et al[40], did a study to study the significance of predictive value of serum ferritin along with Alanine Aminotransferase and glucose levels for assessment of NAFLD with non invasive methods. it demonstrated an association of serum ferritin with liver health as well as glucose and lipid metabolism in NAFLD patients.

Patell R, Dosi R, et al[41], conducted a study with an objective to document the occurrence of NAFLD among obese population in India. Total 60 patients were enrolled in the study and focus was on studying the liver function and various metabolic syndrome components like blood pressure, lipid profile and glycemic index. There was no significant difference in the mean bilirubin values in patients with and without NAFLD whereas mean levels of SGOT and SGPT were found statistically significant. They also concluded that probability of occurrence of NAFLD is higher in obese patients.

Barros K.R et al[42] conducted a study in 2016 in a hospital based in Brazil to study the association of hyperferritinemia in NAFLD patients. It was concluded that hyperferritinemia can be used a tool to check the severity and can help in planning the further management of the patients.

Eguchi et al[43]conducted a study to assess the prevalence and association of metabolic factors of NAFLD in the general population. Total of 5075 patients were enrolled in study. 29.7% were diagnosed with NAFLD, out of which 40% were males. It was concluded that NAFLD has increased in the male population. There is a strong association between metabolic variables and NAFLD.

Shen et al[44]conducted a study to study the prevalence of NAFLD among Shanghai officers. It was seen mainly in patients of 50 years and incidence of fatty liver increased with age. Overweight and hypertriglyceridemia were seen to be associated with fatty liver.

Koehler M et al[45], to check the prevalence and risk factors of NAFLD. 2811 patients participated in the study with a mean age 76.4 ± 6.0 years. NAFLD was seen in 35.1% and was seen to be decreasing with advancing age. There were various potential factors seen like hypertension, diabetes and hypertriglyceridemia.

Fan GJ et al[46] did a study in China to evaluate the prevalence and risk factors of fatty liver. The study included 3175 subjects (1218 men) with a mean age of 52 years. It was seen that males, BMI, triglyceride levels, glucose levels, diabetes and HTN were closely related to fatty liver.

Nomura H, Kashiwagi S et al[47] did a study in Japan to assess the prevalence of fatty liver in the general population. 2574 patients were enrolled in the study and ultrasound abdomen was done on all patients to check for fatty liver. Maximum cases were seen in the age of 40-49 years. Obesity was seen as a very common associated factor in both genders.

Caballeria L et al [48] conducted a study in Spain to check the prevalence of NAFLD and determine the factors associated with severity. 766 patients were included in the study. Mean age of patients was 53+/- 14 years and

showed a male predominance. A multivariate analysis was done and it was seen that NAFLD had a strong association with Male sex, age, components of metabolic syndrome and insulin resistance.

Lu ZY et al[49]conducted a study to investigate the risk factors and occurrence of NAFLD in Chinese population. A total of 1948 patients were included in the study and followed up for 8 years. More than 50 % patients were diagnosed with NAFLD. It was seen that patients with high BMI, fasting glucose levels and high cholesterol had a higher chance of developing NAFLD.

Wang Z et al[50] did a study to assess the prevalence of NAFLD and find associated metabolic factors with NAFLD. It was a case control study done on 4226 patients. Various risk factors were considered like weight of the patient, laboratory investigations and co-morbid conditions and had a strong association with NAFLD.

Chang Y et al[51] conducted a evaluate obese patients not suffering from any metabolic syndrome component and the development of NAFLD in them. 77,425 obese patients were included in the study and were followed up over several years. Ultrasound was done to check the presence of fatty liver. It was observed that with increasing BMI, the incidence of NAFLD increases.

Anstee Q. et al[52] studied the progression of NAFLD towards various metabolic syndrome components. It was shown in their review that NAFLD, not only has hepatic effects but also various extra hepatic consequences mainly DM and abdominal obesity. Thus, NAFLD is a multisystem disorder and complications vary from person to person influenced by both genetic as well as environmental factors.

Liu M et al[53]conducted a retrospective study to determine the relationship between DM and NAFLD in Chinese male. 18,507 males participated in the study and ultrasound was used as diagnostic investigation for NAFLD. Mean age of the patients was 71.38 ± 14.15 years. The participants diagnosed with NAFLD were found to have a higher BMI, hypertension

and hypertriglyceridemia. Incidence of DM was seen comparatively higher in patients with NAFLD. Thus, a strong relationship was established between DM and NAFLD mainly due to BMI variations.

Xiaona Hu et al[54] conducted a study to investigate the prevalence of NAFLD and its association with various risk factors. 7152 individuals underwent a detailed medical work up including history, examination, investigations and abdomen ultrasound. It was seen that 38.17% of the subjects had NAFLD. The disease prevalence increased with the advancing age. A male predominance was seen in the study. Various risk factors were seen to affect the incidence of the disease like BMI, hypertriglyceridemia, HTN and DM.

Trombini P, Piperno A et al[55] studied the association of ferritin, metabolic syndrome and NAFLD. They stated that serum ferritin is commonly seen raised in patients with NAFLD. According to the study, the main cause of raise SF was likely to be a result of a metabolic state or hepatic damage or could be a consequence of NAFLD.

Weismüller T J[56] conducted a study to demonstrate ferritin is a marker for severity of liver disease. They analyzed that patients having a SF >365ng/dl had a higher mortality rate as compared to patients with lower SF. Thus, serum ferritin levels can be used as a predictor of mortality in patients with liver disease and chances of survival after a liver transplant.

# MATERIALS AND METHODS

The present study is a 'prospective observational study' to find out an association of fibrosis on fibroscan and serum ferritin levels, carried out on patients diagnosed with NAFLD coming to the Department of Gastroenterology And Medicine, Dayanand Medical College and Hospital, Ludhiana over a period of one year.

After submission and clearance of the synopsis from the Institutional Ethical Committee, total of 100 patients were randomly and carefully enrolled in the study by applying the inclusion and the exclusion criteria.

**INCLUSION CRITERIA –**Patients diagnosed with NAFLD coming to DMCH for fibro scan will be included in this study.

**EXCLUSION CRITERIA –**

- Autoimmune hepatitis
- Post viral hepatitis
- Anemia (HB<10)
- Wilsons disease
- Haemochromatosis
- Consumption of hepatotoxic drugs
- Decompensated cirrhosis
- Ascites

**METHODOLOGY –**

- A detailed proforma including detailed history, examination and the laboratory data of the patient was recorded for each patient by the primary investigator.
- All necessary investigations including Haemogram, LFTS, viral markers, serum ferritin, USG abdomen, fibroscan were done.
- Patients were evaluated for metabolic syndromes such as obesity, hyperlipidemia and uncontrolled blood sugar levels.
- Levels of serum ferritin were done and measured and a liver elastography was performed to know the degree of fibrosis.

- Degree of fibrosis was based on kpa values.
- Kpa>8.9 was f3 and kpa>11.8 was f4 which were the markers of high grade of fibrosis and f1&f2 were markers of low grade fibrosis
- Liver elastography was done by echo sens fibro scan.

  Data was recorded in the predesigned proforma covering all the above variables.

**OUTCOME**

Degree of fibrosis and serum ferritin levels were correlated in the enrolled NAFLD patients.

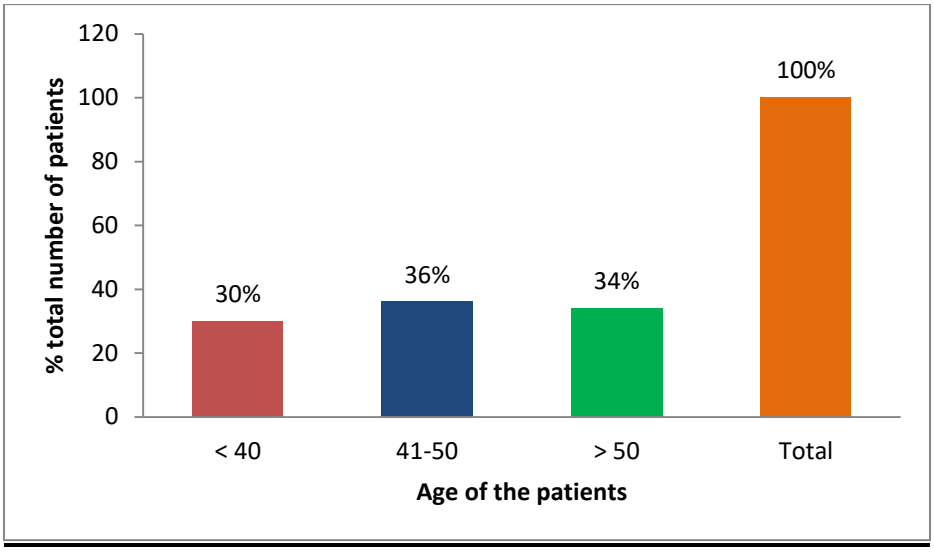
**STATISTICAL ANALYSIS**

Data were described in terms of range; mean ± standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. For comparing categorical data, Chi square ($\chi^2$) test was performed and exact test was used when the expected frequency was less than 5. Pearson correlation was also done to compare the variables. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

# OBSERVATIONS AND RESULTS

Non Alcoholic Fatty Liver Disease is one ofthe emerging liver disease affecting the majority of the population worldwide. NAFLD has been associated with metabolic syndromes like DM, dyslipidemia, obesity. Serum Ferritin being a non invasive, cheap and a readily available biochemical investigation has been used as a screening tool to determine the degree of underlying fibrosis therefore a study was conducted in a tertiary hospital of north india to find a correlation between SF and degree of fibrosis in patients with NAFLD

**Figure 1: Age distribution**

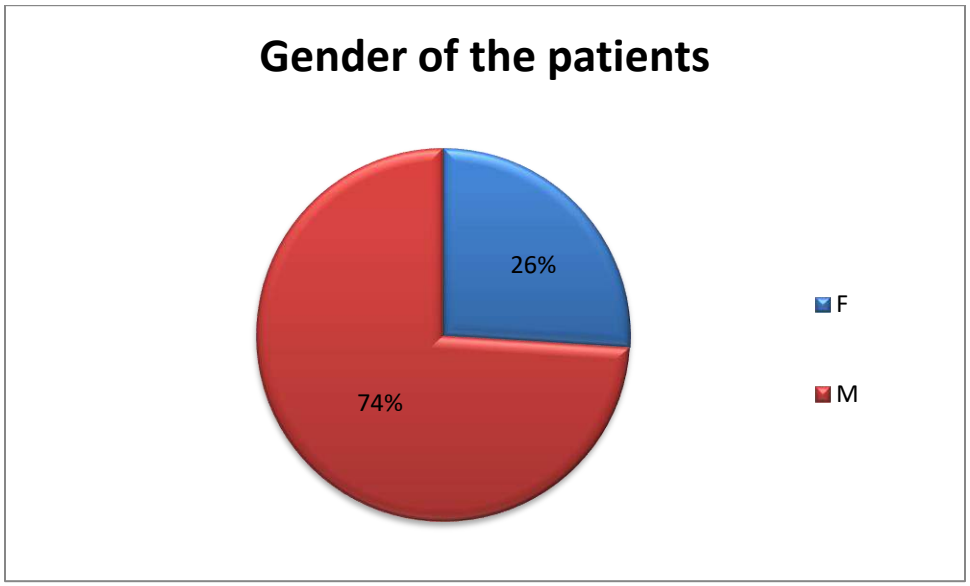



In our study, the mean age of the patients was 45.99 +- 12.17 years. 30% of the patients were <40 years old followed by 36% in the age group 41-50 years and 34% were >50 years. It has been shown in Table 1 and Figure 1.

**Table 1 : Age distribution**

| Age group | No. of cases | Percentage |
|---|---|---|
| < 40 | 30 | 30.0% |
| 41-50 | 36 | 36.0% |
| > 50 | 34 | 34.0% |
| Total | 100 | 100.0% |

**Figure 2: Gender distribution**

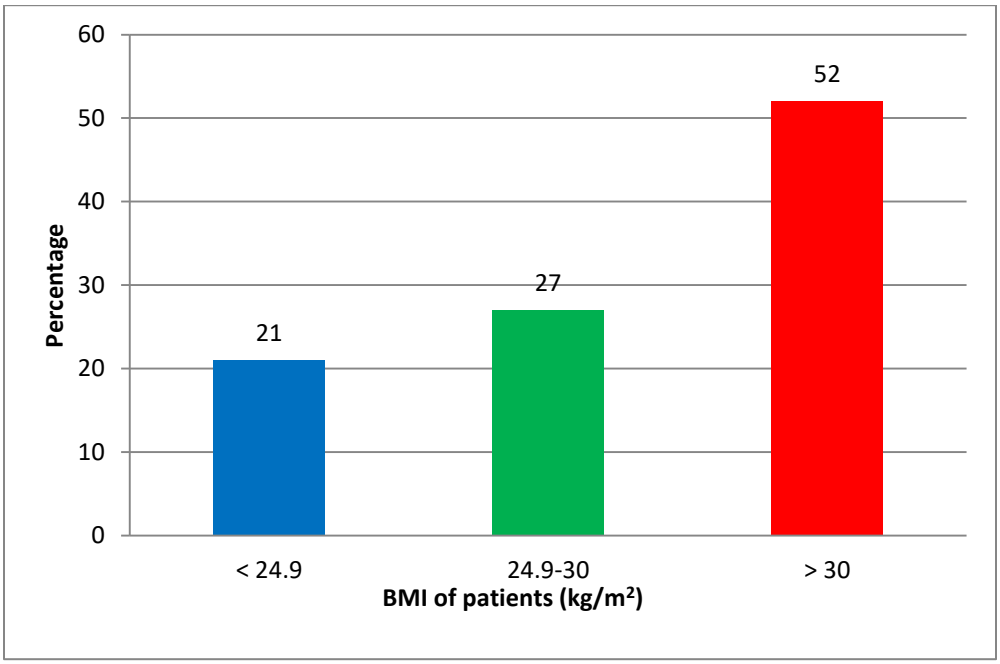



In our study, 74% of the patients were males and 26% were females as described in figure 2 and Table 2.

**Table 2 : Gender distribution**

| Gender | No. of cases | Percentage |
|---|---|---|
| Female | 26 | 26.0% |
| Male | 74 | 74.0% |
| Total | 100 | 100.0% |

**Figure 3: BMI distribution**

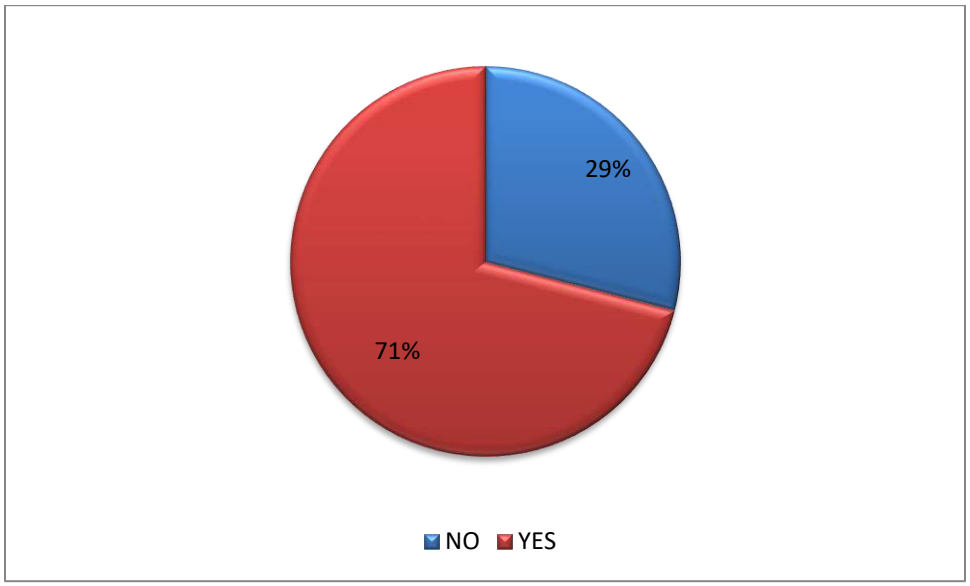



On calculating BMI, majority of the patients were found obese (52%) with a BMI of >30kg/$m^2$ followed by 27 % in overweight category (24.9 to 30 kg/$m^2$) and 21 % with normal BMI as described in figure 3 and table 3.

**Table 3: BMI distribution**

| **BMI GROUP** | No. of cases | Percentage |
|---|---|---|
| < 24.9 | 21 | 21.0% |
| 24.9-30 | 27 | 27.0% |
| > 30 | 52 | 52.0% |
| Total | 100 | 100.0% |

**Figure 4: Diabetes distribution**

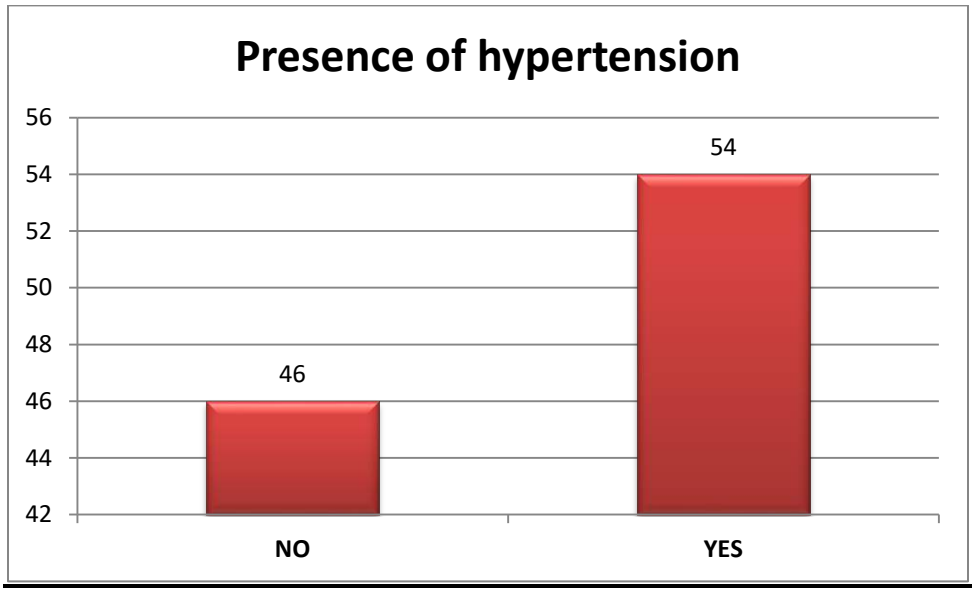



In our study. 71 % patients were diabetic and 29% non-diabetic as shown in figure 4 and table 4.

**Table 4: Diabetes distribution**

| **DIABETES** | No. of cases | Percentage |
|---|---|---|
| NO | 29 | 29.0% |
| YES | 71 | 71.0% |
| Total | 100 | 100.0% |

**Figure 5: Hypertension distribution**

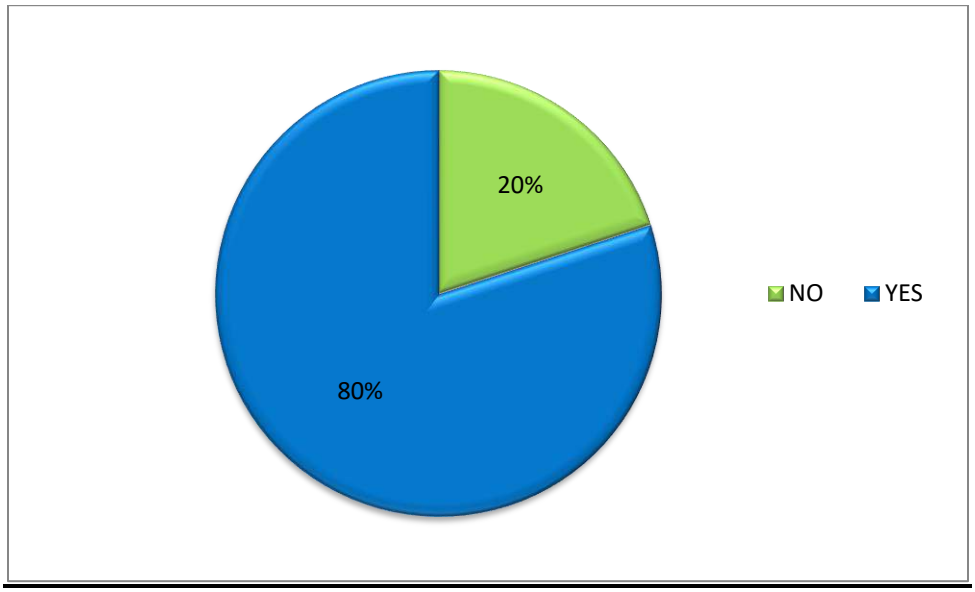



In our study, out of all patients 54% had hypertension whereas 46% were non-hypertensive as described in Figure 5 and table 5.

**Table 5: Hypertension distribution**

| **HTN** | No. of cases | Percentage |
|---|---|---|
| NO | 46 | 46.0% |
| YES | 54 | 54.0% |
| Total | 100 | 100.0% |

**Figure 6: Hypertriglyceridemia distribution**

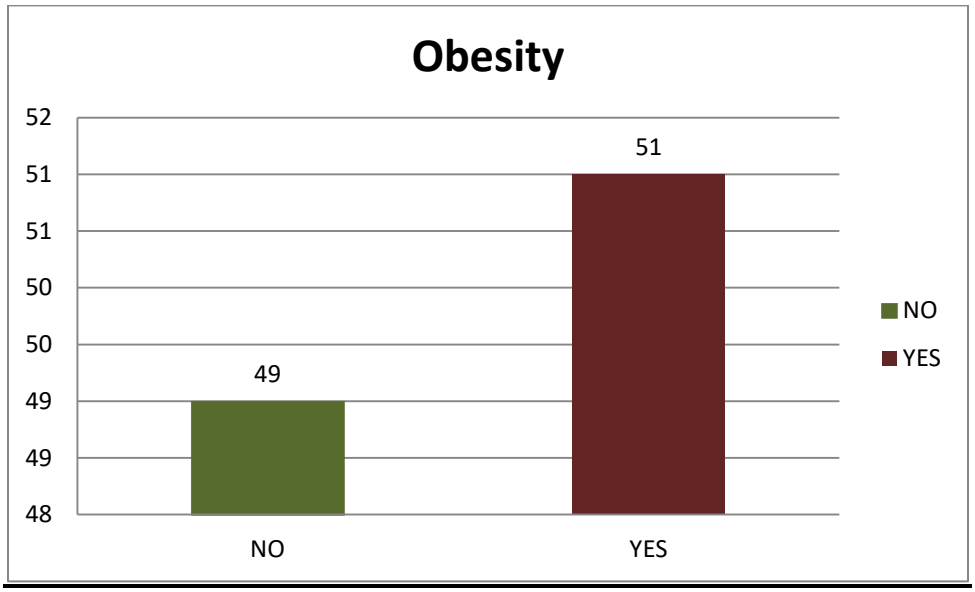



After investigating, it was seen that 80% patients had high levels of triglycerides whereas 20% had normal levels as described in figure 6 and table 6.

**Table 6: Hypertriglyceridemia distribution**

| **HYPERTRIGLYCERDEMIA** | No. of cases | Percentage |
|---|---|---|
| NO | 20 | 20.0% |
| YES | 80 | 80.0% |
| Total | 100 | 100.0% |

**Figure 7: Obesity distribution**

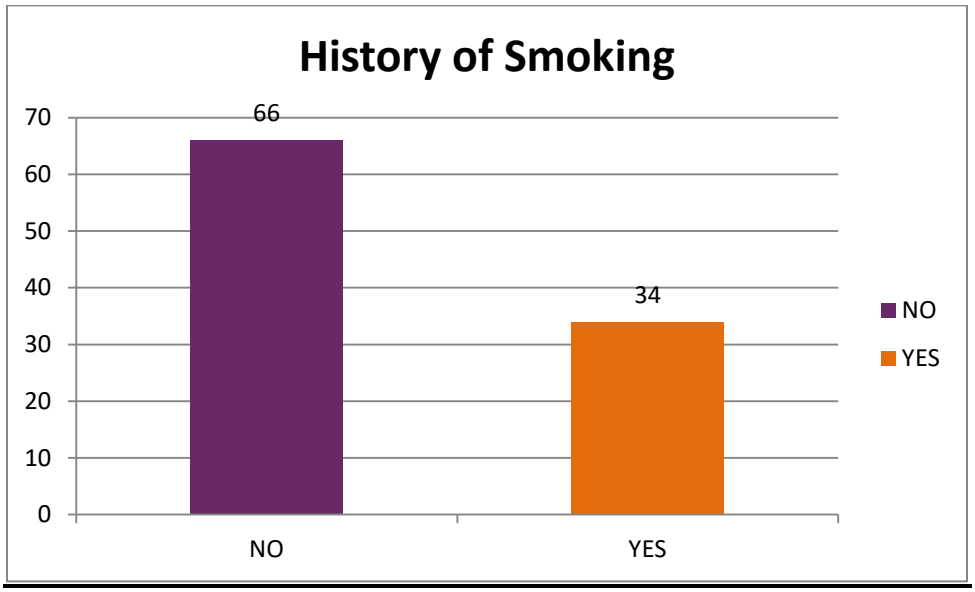



In our study, 51% of the patients were obese and 49% were not obese as shown in figure 7 and table 7.

**Table 7: Obesity distribution**

| **OBESITY** | No. of cases | Percentage |
|---|---|---|
| NO | 49 | 49.0% |
| YES | 51 | 51.0% |
| Total | 100 | 100.0% |

**Figure 8: Smoking distribution**

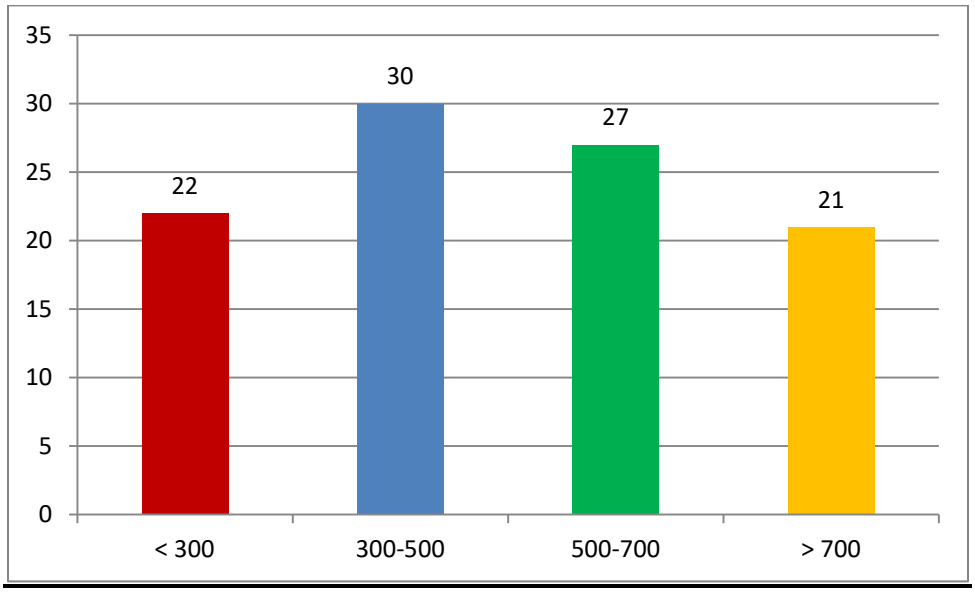



In our study, 34% patients gave a history of smoking and 66% were non-smokers as shown in figure 8 and table 8.

**Table 8: Smoking distribution**

| **SMOKING** | No. of cases | Percentage |
|---|---|---|
| NO | 66 | 66.0% |
| YES | 34 | 34.0% |
| Total | 100 | 100.0% |

**Figure 9: Ferritin levels distribution**

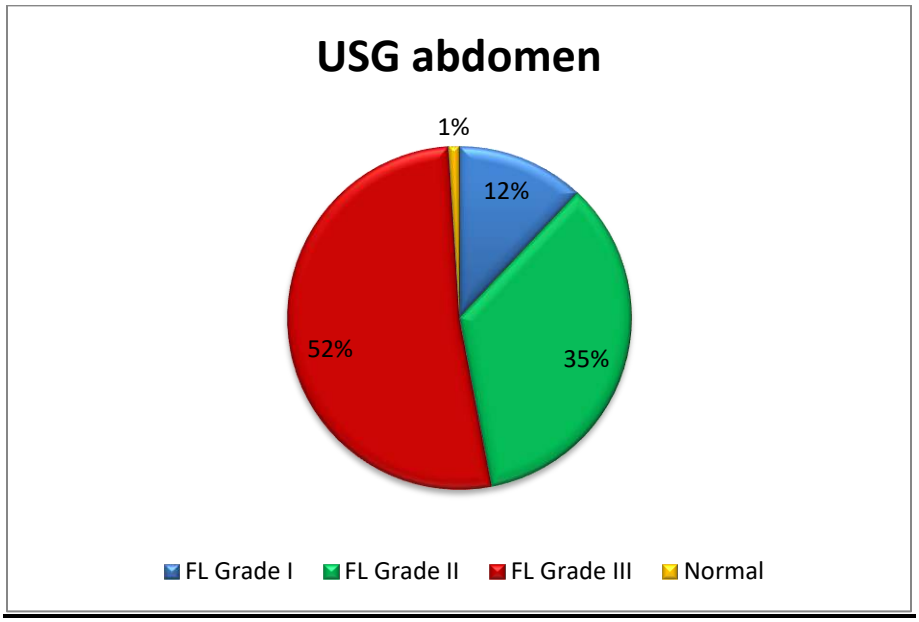



In our study, majority of patients (30%) had Serum ferritin levels between 300-500ng/dl, followed by 27% havingSF in the range 500-700ng/dl, 21% having SF >700ng/dland the rest 22% had normal SF as described in figure 8 and table 8.

**Table 9: Ferritin levels distribution**

| **Ferritin group** | No. of cases | Percentage |
|---|---|---|
| < 300 | 22 | 22.0% |
| 300-500 | 30 | 30.0% |
| 500-700 | 27 | 27.0% |
| > 700 | 21 | 21.0% |
| Total | 100 | 100.0% |

**Figure 10: USG abdomen distribution**

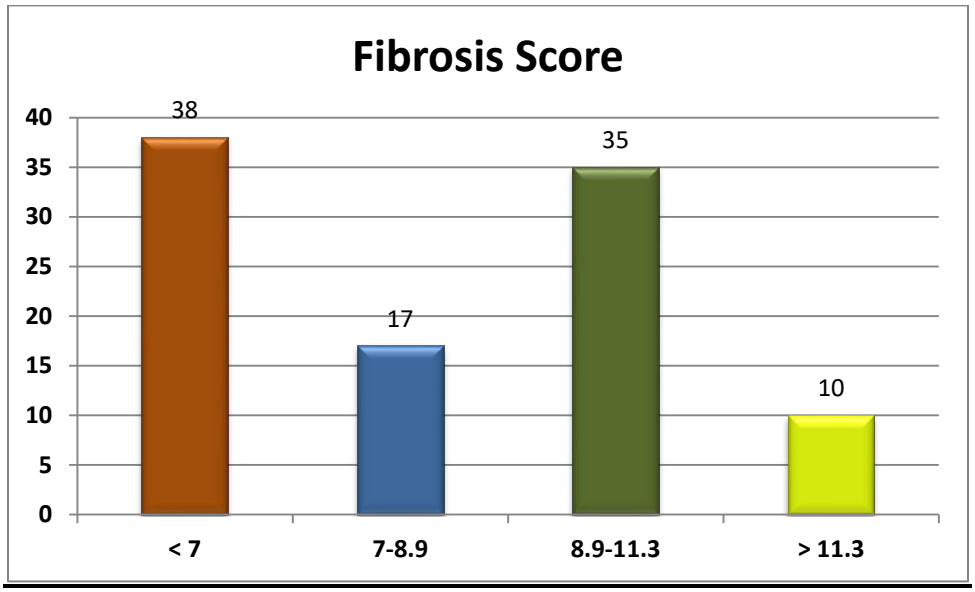



In our study, the ultrasound abdomen results showed that 52% patients had FL Grade III, 35% had FL Grade II, 12% had FL Grade I and 1% showed normal report as seen in Figure 9 and table 9.

**Table 10: USG abdomen distribution**

| **USG AB** | No. of cases | Percentage |
|---|---|---|
| FL Grade I | 12 | 12.0% |
| FL Grade II | 35 | 35.0% |
| FL Grade III | 52 | 52.0% |
| Normal | 1 | 1.0% |
| Total | 100 | 100.0% |

**Figure 11 : Fibrosis Score Distribution**

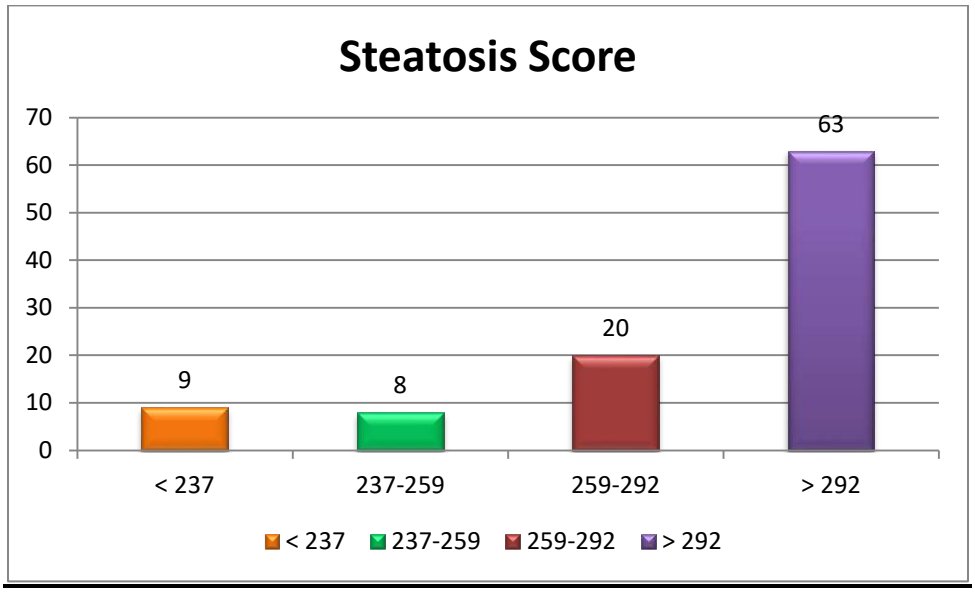



The study showed that 38% patients had a fibrosis score of <7kPa, followed by 35% having a score between 8.9-11.3kPa and 17% with a score of 7-8.9kPa and only 10 % with fibrosis score of >11.3kPa.
It has been depicted in figure 10 and table 10.

**Table 11: Fibrosis Score Distribution**

| **FIBRROSIS SCORE group** | No. of cases | Percentage |
|---|---|---|
| < 7 | 38 | 38.0% |
| 7-8.9 | 17 | 17.0% |
| 8.9-11.3 | 35 | 35.0% |
| > 11.3 | 10 | 10.0% |
| Total | 100 | 100.0% |

**Figure 12: Steatosis Score Distribution**




In our study, majority of patients comprising of 63% had a steatosis score >292. It was followed by 20% having a score of 259-292, similar percentage of patients 9% and 8% having a score of <237 and 237-259 respectively as mentioned in table 11 and figure11.

**Table 12: Steatosis Score Distribution**

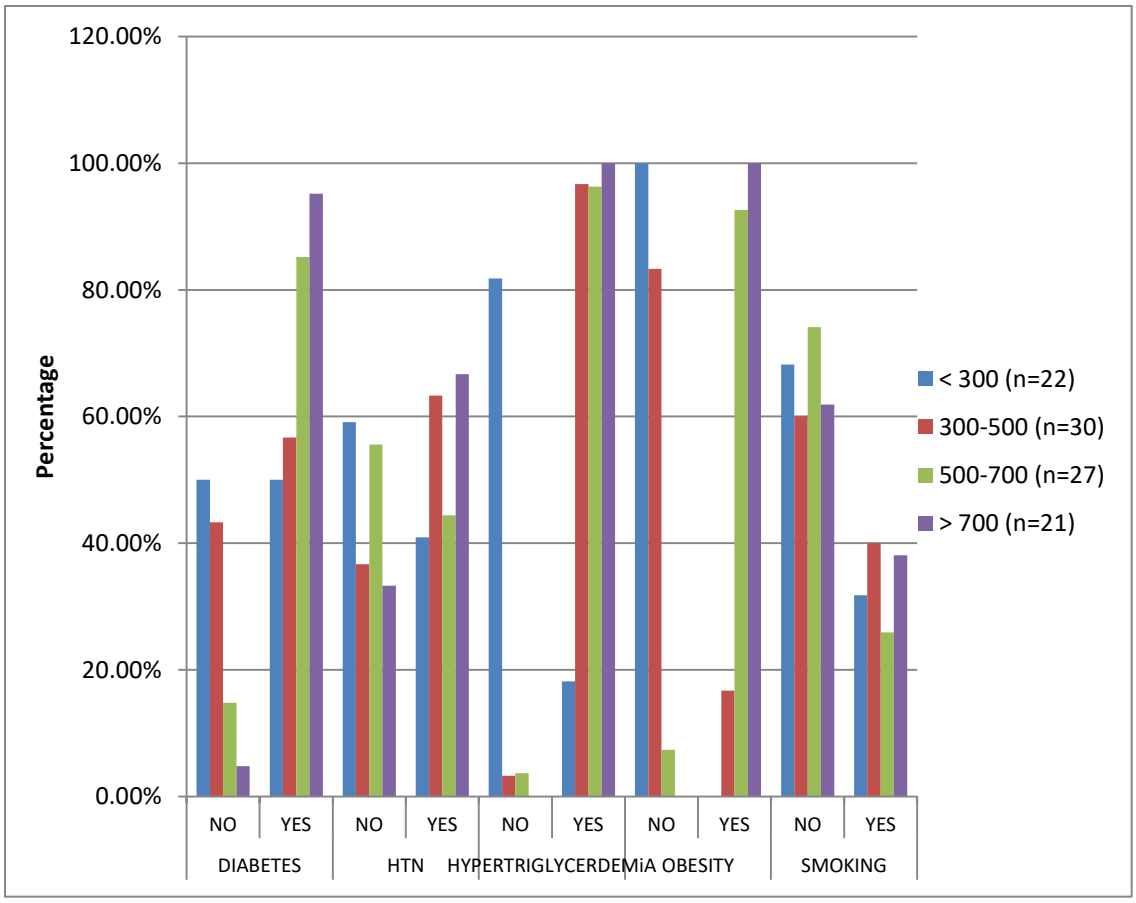
| **STEATOSIS SCORE group** | No. of cases | Percentage |
|---|---|---|
| < 237 | 9 | 9.0% |
| 237-259 | 8 | 8.0% |
| 259-292 | 20 | 20.0% |
| > 292 | 63 | 63.0% |
| Total | 100 | 100.0% |

**Table 13 : Mean distribution of various parameters in our study**

| | N | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|---|
| AGE | 100 | 25.00 | 76.00 | 45.99 | 12.17 |
| BMI | 100 | 20.20 | 41.10 | 29.91 | 5.76 |
| Hb | 100 | 10.10 | 14.60 | 11.72 | 1.05 |
| BILIRUBIN | 100 | 0.30 | 2.10 | 1.09 | 0.40 |
| SGOT | 100 | 16.00 | 110.00 | 55.97 | 18.83 |
| SGPT | 100 | 40.00 | 280.00 | 107.09 | 50.65 |
| FERRITIN | 100 | 72.20 | 900.60 | 478.97 | 216.57 |
| FIBRROSIS SCORE | 100 | 2.13 | 13.20 | 8.02 | 2.70 |
| STEATOSIS SCORE | 100 | 146.00 | 400.00 | 300.14 | 47.65 |
| HBA1C | 76 | 5.20 | 13.20 | 8.11 | 1.78 |

In our study, amongst a total of 100 patients, the mean age observed was 45.99 ± 12.17 years. Mean ferritin value obtained was 478.97 ± 216.57. Fibroscan was done to check the severity of NAFLD and it was concluded that mean value of fibrosis score and steatosis score was 8.02 ± 2.70 and 300 ± 47.65 respectively. Mean values of various other biochemical parameters were as mentioned in Table 12 above.

**Figure 13- Univariate analysis of ferritin and predisposing factors:**




In our study, a univariate analysis was done to correlate the SF levels with underlying predisposing factors like DM, HTN, hypertriglyceridemia, obesity and smoking. Out of 100 patients enrolled in the study, 71% patients were having diabetes and amongst these 11 patients had a normal SF, 17 patients had SF between 300-500ng/dl, 23 patients had a SF between 500-700ng/dl and the rest 20 patients had a SF value >700ng/dl. Thus, it was seen that patients with diabetes had increased SF values which was statistically significant ( p- value -0.001)

It was seen in the study, 54 patients were hypertensive, out of which 19 patients had SF between 300-500ng/dl followed by 14 patients having SF >700ng/dl, 12 patients with SF between 500-700ng/dl and the rest 9

patients had normal values. It was seen that there is no statistical significance in the two parameters.( p-value 0.178)

In our study, 80% patients had hypertriglyceridemia. Majority of the patients had higher ferritin values whereas only 5% of these had normal SF levels. Statistical significance was seen (p-value 0.001) amongst the two parameters.

In our study, 51 patients had obesity. Out of these, 25 patients had SF between 500-700ng/dl, 21 patients showed ferritin values >700ng/dl and 5 patients had SF 300-500ng/dl. Thus, majority of obese patients had higher ferritin values which was statistically significant ( p-value 0.0001).

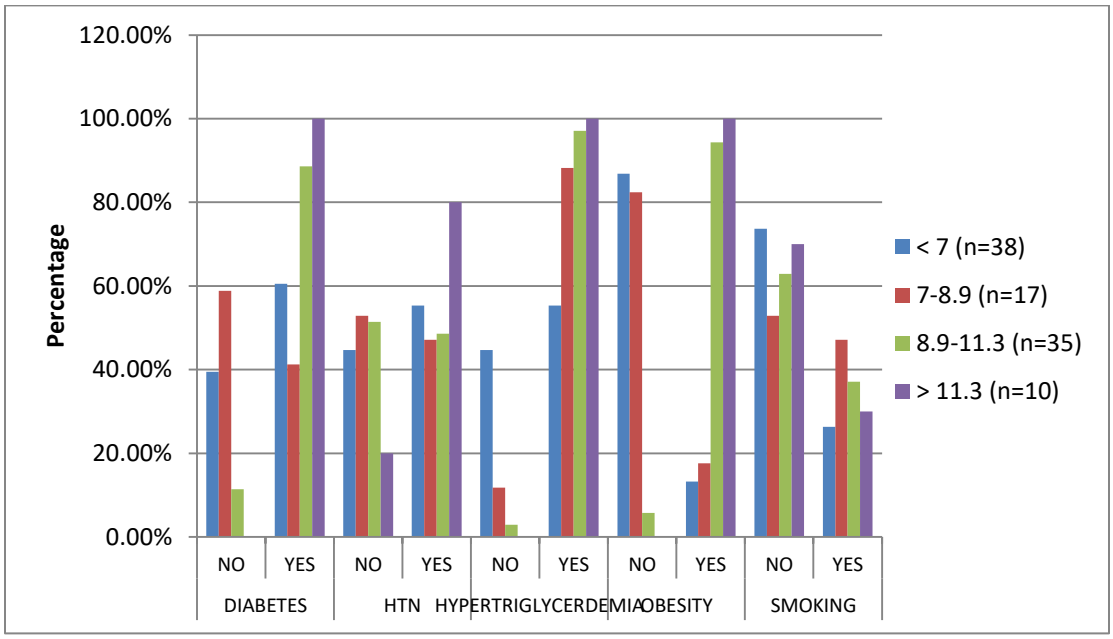
Smoking showed no correlation with ferritin values (p-value 0.689)

This has been mentioned in Table 14.

**Table 14: Univariate analysis of ferritin and predisposing factors**

| | | Ferritin group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 300 (n=22) | | 300-500 (n=30) | | 500-700 (n=27) | | > 700 (n=21) | | | | |
| DIABETES | NO | 11 | 50.0% | 13 | 43.3% | 4 | 14.8% | 1 | 4.8% | 29 | 16.336 | 0.001 |
| | YES | 11 | 50.0% | 17 | 56.7% | 23 | 85.2% | 20 | 95.2% | 71 | | |
| HTN | NO | 13 | 59.1% | 11 | 36.7% | 15 | 55.6% | 7 | 33.3% | 46 | 4.919 | 0.178 |
| | YES | 9 | 40.9% | 19 | 63.3% | 12 | 44.4% | 14 | 66.7% | 54 | | |
| HYPERTRIGLYCERDEMIA | NO | 18 | 81.8% | 1 | 3.3% | 1 | 3.7% | 0 | 0.0% | 20 | 67.485 | 0.0001 |
| | YES | 4 | 18.2% | 29 | 96.7% | 26 | 96.3% | 21 | 100.0% | 80 | | |
| OBESITY | NO | 22 | 100.0% | 25 | 83.3% | 2 | 7.4% | 0 | 0.0% | 49 | 75.916 | 0.0001 |
| | YES | 0 | 0.0% | 5 | 16.7% | 25 | 92.6% | 21 | 100.0% | 51 | | |
| SMOKING | NO | 15 | 68.2% | 18 | 60.0% | 20 | 74.1% | 13 | 61.9% | 66 | 1.469 | 0.689 |
| | YES | 7 | 31.8% | 12 | 40.0% | 7 | 25.9% | 8 | 38.1% | 34 | | |

**Figure14- Univariate analysis of Fibrosis and predisposing factors**




In this study 71 patients had diabetes, out of which 23 patients had a Fibrosis score F1, 7 patients had F2 fibrosis score, majority of patients had a fibrosis score of F3 (31%) and rest 10 patients had a fibrosis score of F4. Two – thirds of the diabetic patients had higher grade of fibrosis which was statistically significant (p-value 0.001) as mentioned in the table 17.

In our study, 54 patients had hypertension. Out of these, 21 patients had F1 fibrosis, 8 patients had F2 fibrosis, 17 and 8 patients had a fibrosis score of F3 and F4 respectively. Thus, it was seen that hypertension and degree of fibrosis had no correlation and was not statistically significant (p-value 0.322)

It was seen that 80 patients had hypertrigyceridemia. Maximum patients out of these fell in the category of F3 fibrosis score(34 patients) followed by F1 fibrosis score (21 patients), F2 score (15 patients) and F4 score (10 patients). Hypertriglyceridmeia was a common finding in the study and it was seen that patients with hypertriglyceridemia had an underlying fibrosis

which was statistically significant as mentioned in the table (p-value 0.001).

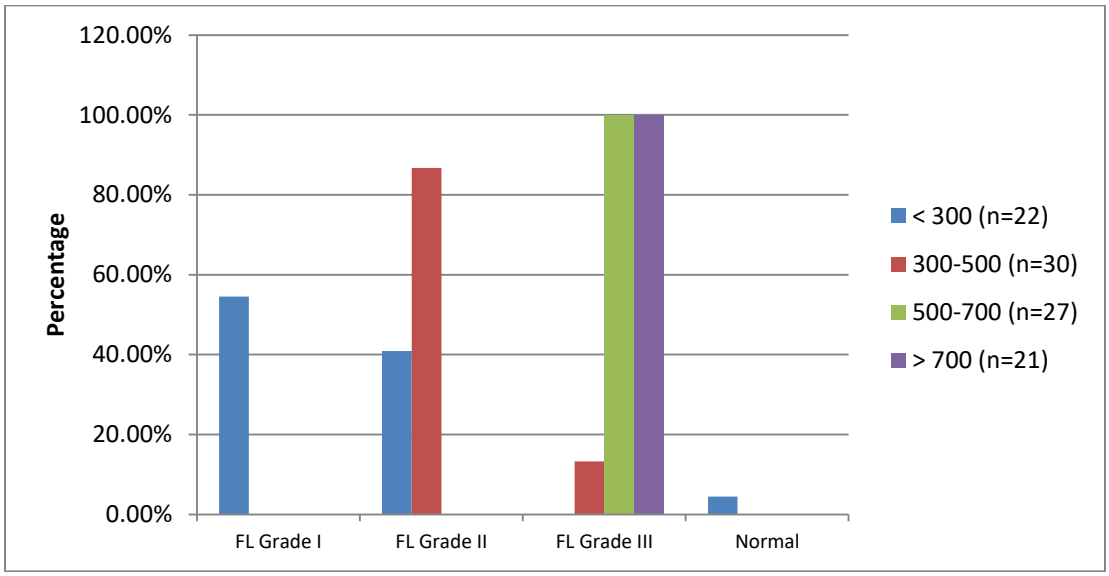
Obesity was observed in 52 patients and majority of them had F3 and F4 fibrosis on fibroscan. It was observed that patient with a higher BMI had higher degree of fibrosis which was statistically significant. (p-value 0.001)

In our study, 34 patients had a history of smoking. Out of these, 18 patients had low grade fibrosis (F1 and F2) and the rest 16 patients had higher grade fibrosis (F3 and F4). It showed that smoking had no effect on the degree of fibrosis and was not statistically significant. (p-value 0.472)

**Table 15: Univariate analysis of fibrosis score with predisposing factors**

| | | FIBRROSIS SCORE group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 7 (n=38) | | 7-8.9 (n=17) | | 8.9-11.3 (n=35) | | > 11.3 (n=10) | | | | |
| DIABETES | NO | 15 | 39.5% | 10 | 58.8% | 4 | 11.4% | 0 | 0.0% | 29 | 18.701 | 0.0001 |
| | YES | 23 | 60.5% | 7 | 41.2% | 31 | 88.6% | 10 | 100.0% | 71 | | |
| HTN | NO | 17 | 44.7% | 9 | 52.9% | 18 | 51.4% | 2 | 20.0% | 46 | 3.491 | 0.322 |
| | YES | 21 | 55.3% | 8 | 47.1% | 17 | 48.6% | 8 | 80.0% | 54 | | |
| HYPERTRIGLYCERIDEMIA | NO | 17 | 44.7% | 2 | 11.8% | 1 | 2.9% | 0 | 0.0% | 20 | 24.182 | 0.001 |
| | YES | 21 | 55.3% | 15 | 88.2% | 34 | 97.1% | 10 | 100.0% | 80 | | |
| OBESITY | NO | 33 | 86.8% | 14 | 82.4% | 2 | 5.7% | 0 | 0.0% | 49 | 65.192 | 0.001 |
| | YES | 5 | 13.2% | 3 | 17.6% | 33 | 94.3% | 10 | 100.0% | 51 | | |
| SMOKING | NO | 28 | 73.7% | 9 | 52.9% | 22 | 62.9% | 7 | 70.0% | 66 | 2.517 | 0.472 |
| | YES | 10 | 26.3% | 8 | 47.1% | 13 | 37.1% | 3 | 30.0% | 34 | | |

**Figure 15- Correlation between ultrasound abdomen findings and serum ferritin**

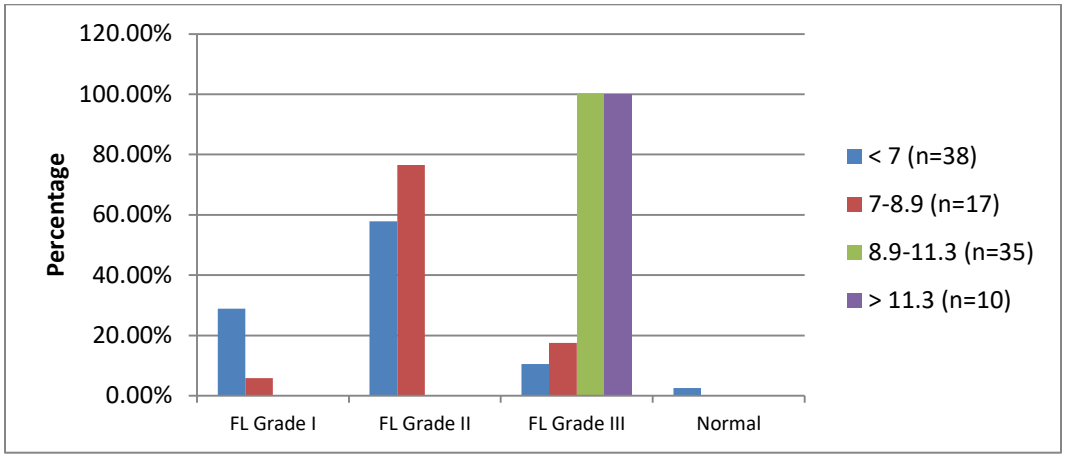



In our study of 100 patients, 22 patients had normal SF levels. Out of these, majority had FL Grade I (54.5%) on USG abdomen. 30 patients had SF between 300-500ng/dl, out of which 86.7% had FL Grade II. All patients having ferritin levels >500ng/dl had a FL Grade III on ultrasound. There was statistical significance between elevated serum ferritin values with severity of fatty liver as diagnosed with ultrasound abdomen (p-value 0.0001).

**Table 16: Correlation between ultrasound abdomen findings and serum ferritin**

| | | Ferritin group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 300 (n=22) | | 300-500 (n=30) | | 500-700 (n=27) | | > 700 (n=21) | | | | |
| **USG AB** | FL Grade I | 12 | 54.5% | 0 | 0.0% | 0 | 0.0% | 0 | 0.0% | 12 | 127.325 | 0.0001 |
| | FL Grade II | 9 | 40.9% | 26 | 86.7% | 0 | 0.0% | 0 | 0.0% | 35 | | |
| | FL Grade III | 0 | 0.0% | 4 | 13.3% | 27 | 100.0% | 21 | 100.0% | 52 | | |
| | Normal | 1 | 4.5% | 0 | 0.0% | 0 | 0.0% | 0 | 0.0% | 1 | | |

**Figure 16- Correlation of USG Abdomen findings and degree of fibrosis on fibroscan**

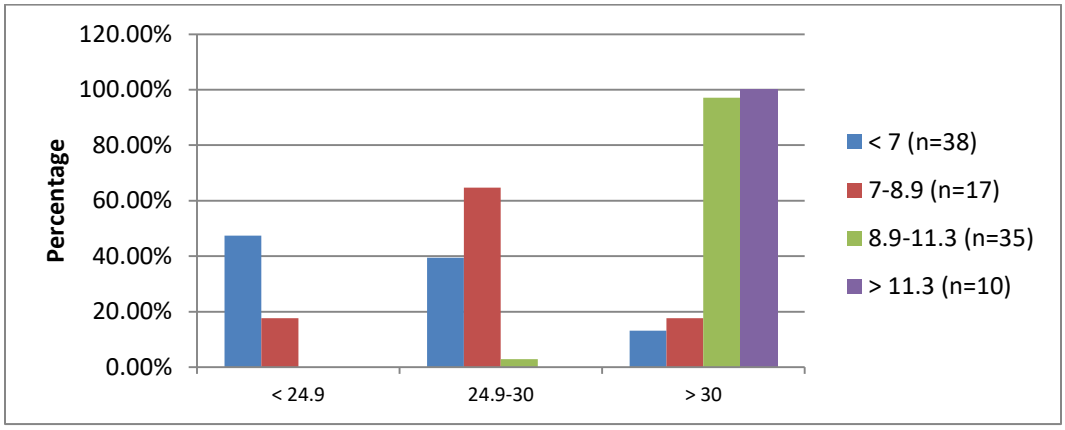



In our study, Majority of the patients with FL Grade 1 had F1 fibrosis (11 out of 12 patients). 35 patients had FL Grade II of which 22 patients had F1 fibrosis and 13 patients had F2 fibrosis. 45 patients of the 52 patients having FL Grade III, had higher degree of fibrosis. Thus, it was seen that the sonological findings correlated with the fibrosis score obtained on fibroscan and was statistically significant. (p-value 0.001)

**Table 17: Correlation of USG Abdomen findings and degree of fibrosis on fibroscan**

| | | FIBRROSIS SCORE group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 7 (n=38) | | 7-8.9 (n=17) | | 8.9-11.3 (n=35) | | > 11.3 (n=10) | | | | |
| USG AB | FL Grade I | 11 | 28.9% | 1 | 5.9% | 0 | 0.0% | 0 | 0.0% | 12 | 82.817 | 0.0001 |
| | FL Grade II | 22 | 57.9% | 13 | 76.5% | 0 | 0.0% | 0 | 0.0% | 35 | | |
| | FL Grade III | 4 | 10.5% | 3 | 17.6% | 35 | 100.0% | 10 | 100.0% | 52 | | |
| | Normal | 1 | 2.6% | 0 | 0.0% | 0 | 0.0% | 0 | 0.0% | 1 | | |

**Figure 17- Correlation between BMI and Fibrosis score:**

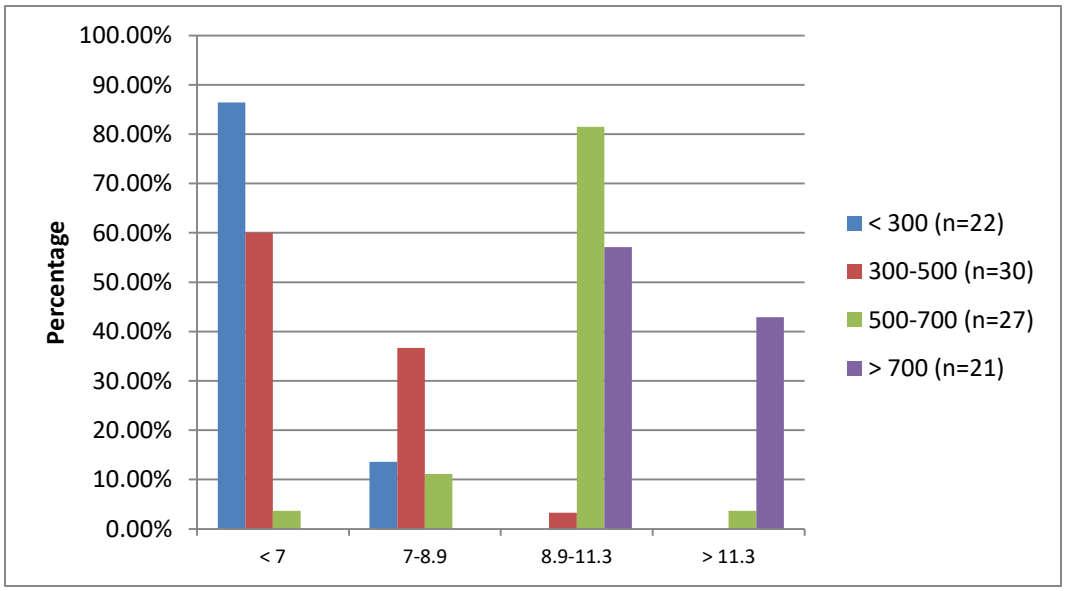



In our study the fibrosis score was compared to BMI distribution .A total of 21 patients had a normal BMI, out of which 18 patients had F1 fibrosis,27 patients were overweight and 15 of them had F1 fibrosis ,11 patients has F2 fibrosis.Obesity was seen in 52 patients, majority of them had F3 and F4 fibrosis on fibroscan.It was observed that patient with a higher BMI had higher degree of fibrosis which was statistically significant. (p-value 0.0001)

**Table 18 – Correlation between BMI and Fibrosis score**

| | | FIBRROSIS SCORE group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 7 (n=38) | | 7-8.9 (n=17) | | 8.9-11.3 (n=35) | | > 11.3 (n=10) | | | | |
| BMI GROUP | < 24.9 | 18 | 47.4% | 3 | 17.6% | 0 | 0.0% | 0 | 0.0% | 21 | 76.55 | 0.0001 |
| | 24.9-30 | 15 | 39.5% | 11 | 64.7% | 1 | 2.9% | 0 | 0.0% | 27 | | |
| | > 30 | 5 | 13.2% | 3 | 17.6% | 34 | 97.1% | 10 | 100.0% | 52 | | |

**Figure 18- Correlation between fibrosis score and ferritin**




Fibroscan was done in the patients to assess the severity of NAFLD and the fibrosis score obtained was compared with the SF levels. Patients were divided into 4 groups according to the fibrosis score: F1 (<7 kPa), F2 ( 7-8.9 kPa), F3 (8.9-11.3 kPa) and F4 (>11.3 kPa). It was seen that 38 patients had a F1 Fibrosis score of which 19 patients had normal ferritin value and the rest 18 patients had a SF value in the range 300-500ng/dl. Out of the 17 patients having F2 fibrosis score, 11 patients had SF between 300-500ng/dl. A total of 35 patients had F3 fibrosis out of which majority of patients (81.5%) had SF between 500-700ng/dl, 10 patients had F4 fibrosis score and out of these 9 patients had SF >700ng/dl with a maximum SF of 900.6ng/dl. It was seen that patients with higher degree of fibrosis had a higher serum ferritin value which was statistically significant (p-value 0.001).

**Table 19: Correlation between fibrosis score and ferritin**

| | | Ferritin group | | | | | | | | Total | Chi-square value | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | < 300 (n=22) | | 300-500 (n=30) | | 500-700 (n=27) | | > 700 (n=21) | | | | |
| FIBRROSIS SCORE group | < 7 | 19 | 86.4% | 18 | 60.0% | 1 | 3.7% | 0 | 0.0% | 38 | 109.639 | 0.0001 |
| | 7-8.9 | 3 | 13.6% | 11 | 36.7% | 3 | 11.1% | 0 | 0.0% | 17 | | |
| | 8.9-11.3 | 0 | 0.0% | 1 | 3.3% | 22 | 81.5% | 12 | 57.1% | 35 | | |
| | > 11.3 | 0 | 0.0% | 0 | 0.0% | 1 | 3.7% | 9 | 42.9% | 10 | | |

**Table 20: Correlation of various variables in the study:**

| | | AGE | BMI | HBA1C | Hb | BILIRUBIN | SGOT | SGPT | FERRITIN | FIBRROSIS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|
| **BMI** | Pearson Correlation | -0.112 | 1.000 | 0.710 | 0.071 | 0.708 | 0.493 | 0.626 | 0.727 | 0.720 |
| | p-value | 0.268 | | 0.000 | 0.483 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | N | 100 | 100 | 76 | 100 | 100 | 100 | 100 | 100 | 100 |
| **SGOT** | Pearson Correlation | 0.062 | 0.493 | 0.417 | 0.093 | 0.548 | 1.000 | 0.706 | 0.512 | 0.469 |
| | p-value | 0.537 | 0.000 | 0.000 | 0.357 | 0.000 | | 0.000 | 0.000 | 0.000 |
| | N | 100 | 100 | 76 | 100 | 100 | 100 | 100 | 100 | 100 |
| **SGPT** | Pearson Correlation | 0.029 | 0.626 | 0.464 | 0.060 | 0.627 | 0.706 | 1.000 | 0.633 | 0.587 |
| | p-value | 0.774 | 0.000 | 0.000 | 0.552 | 0.000 | 0.000 | | 0.000 | 0.000 |
| | N | 100 | 100 | 76 | 100 | 100 | 100 | 100 | 100 | 100 |
| **FERRITIN** | Pearson Correlation | 0.031 | 0.756 | 0.599 | 0.123 | 0.666 | 0.493 | 0.610 | 1.000 | 0.897 |
| | p-value | 0.758 | 0.000 | 0.000 | 0.221 | 0.000 | 0.000 | 0.000 | | 0.000 |
| | N | 100 | 100 | 76 | 100 | 100 | 100 | 100 | 100 | 100 |

A correlation analysis was done for all variables included in the study.It was found that BMI has an association with HBA1C,SGOT/SGPT, ferritin and fibrosis score out of which BMI has the strongest corealtion with Ferritin (Pearson correlation value 0.727). Ferritin had a statically significant correlation with different variables included in the study amongst which it was found to have the strongest association with fibrosis score (Pearson correlation value0.897) Liver enzymes SGOT/SGPT were observed to have a significant association with BMI, serum ferritin, fibrosis score and HBA1C but was most significantly corelated with ferritin values. (Pearson correlation value 0.512 and 0.633 respectively).

# DISCUSSION

NAFLD is recognized as the leading cause of chronic liver disease worldwide, and, in a percentage of cases, it causes advanced fibrosis and severe complications. As a consequence, the need for predictive factors of NAFLD and especially of its progressive forms is mandatory. Ferritin has been seen as an important marker to assess the severity of the disease. Being a non-invasive, cheap and readily available biochemical investigation, various studies have been done to demonstrate its accuracy as an alternative diagnostic tool for degree of fibrosis in NAFLD. Thus, this study was conducted to find the correlation of ferritin levels and fibroscan with the severity of the disease.

A prospective observational study was conducted in a tertiary care hospital of North India involving 100 patient diagnosed with NAFLD to find an association between SF levels and grades of fibrosis and also to define values of SF which could differentiate the degree of fibrosis. In this study the possible risk factors for metabolic syndrome and insulin resistance were taken into consideration like obesity, dyslipidemia, DM, smoking. The statistical data obtained from this study was compared with the previously published literatures.

Majority of the patients belonged to the age group of 40-49 years (36%), followed by age group of >50 years having almost equal percentage of 34% patients. The mean age was 45.99 +- 12.17 years. Thus it suggested that NAFLD is common after the age of 40 years and is less prevalent in younger population. It was the same as found by Eguchi et al[43] who observed that maximum patients diagnosed with NAFLD were in the age group of 40-49 years., Shan et al[44] who conducted a study in patients 20-81 years of age and observed that maximum patients were >50 years of age, Nomura et al[47] who conducted a study in Japanese population from the age group of 0-70 years, it was found that only 1.2% patients were less than 19 years of age and maximum patients were in age group 40-49 years.

In present study, there was a male predominance with 74 % males and females comprising of 26%. This was similar to that seen in studies conducted by Caballeria et al[48], Lu ZY et al[49] and Wang et al[50] which showed male gender was associated with incidence of fatty liver. No statistical significance was seen between gender of the patient and fibrosis score (p value 0.832) as well as with serum ferritin levels (p value 0.768).

In our study, out of all 100 patients, 52% cases were obese patients (BMI >30 kg/$m^2$) followed by 27% who were overweight (BMI 24.9– 29.9kg/$m^{2.}$). Rest 21% cases had normal weight. The study showed statistical significance of BMI with severity of NAFLD based on fibrosis score (p value 0.001) and also with increased SF values (p value 0.001). These results were similar to those mentioned in various previous studies like .Manousou P et al[5] which showed strong association between BMI and severity of fibrosis. Chang et al[51] conducted that increased BMI had a linear relationship with incidence of NAFLD.

In this study 71% patients had diabetes, 51% patients were obese and 80% patients had underlying hypertriglyceridemia. Fibroscan done on 100 patients showed that 55% of patients had high grade fibrosis and rest 45% had low grade fibrosis. Liu M et al[53] ,Vernon G et al[36] and Anstee M Q et al[52] also showed similar results and concluded that majority of patient with NAFLD had underlying diabetes. Moghaddasifar et al[27] and Xiaona Hu et al[54]also demonstrated the prevalence of metabolic syndromes in occurance of NAFLD and found that majority of patient included in the study had DM2,obesity & dyslipidemia.

In our study 21% patients had a normal BMI of which majority of the patients had a normal SF. 27% patients were overweight of which maximum patients had a SF in the range 300-500ng/dl. Obesity was found in 52% patients and majority of them had a SF >500ng/dl. A study conducted by Alam *et al.* [17] on 150 patients in the age group of 20-60 years concluded that obesity led to hyperferritinemia irrespective of actual

body iron stores. Out of 100 patients enrolled in this study, 71% patients had diabetes and maximum patients had a SF between 500-700ng/dl. Ford et al.[1] and Salonen et al(2) also concluded that patients with hyperferritinemia had a 2.4-fold higher risk of developing T2DM.

We observed, 80% patients had hypertriglyceridemia. Majority of the patients had higher ferritin levels whereas only 5% (4 out of 80 patients) had normal SF levels. Statistical significance was seen (p-value 0.001) amongst the two parameters. Manousou P, Kalambokis G et al[5], conducted a study on 111 patients with NAFLD, out of which 40% had hyperlipdemia and majority of the patients had a higher serum ferritin levels.

**Association of serum ferritin with fibrosis score-**

In our study it was observed that 38 patients had a F1 Fibrosis score of which 19 patients had normal ferritin value and the rest 18 patients had a SF value in the range 300-500ng/dl. Out of the 17 patients having F2 score, 11 patients had SF between 300-500ng/dl. A total of 35 patients had fibrosis F3, majority of patients (81.5%) had SF between 500-700ng/dl. 10 patients had F4 fibrosis score and out of these 9 patients had SF >700ng/dl with a maximum SF of 900.6ng/dl. It was concluded that patients with higher SF levels had a higher degree of fibrosis on Fibroscan which was statistically significant (p-value 0.001). SF levels of more than 500ng/ml showed higher degree of fibrosis (F3 and F4) whereas ferritin levels below 500ng/ml were likely to have mild fibrosis. In other studies conducted by Seyedian S et al(28) and Kowdley et al(6), the mean serum ferritin levels showing higher grade of fibrosis were 225ng/ml and 450ng/ml respectively.

Seyedian S et al(28) conducted a study to establish a relationship between ferritin values and findings on fibroscan among patients of NAFLD. Patients were categorized into mainly two groups on the basis of liver stiffness obtained on fibroscan, mild liver stiffness (F0 – F2, liver stiffness measurement (LSM) <8.7) and advanced liver stiffness (F3, F4, LSM

≥8.7). Out of 284 patients included in the study, 226 patients had mild liver stiffness whereas 58 patients had advanced liver stiffness. The average level of serum ferritin in mild liver stiffness group and in advanced liver stiffness group was 132 ± 101/6ng/dl and 222/8 ± 194.4ng/dl respectively which showed a statistical significance (P < 0.001).

Furthermore, studies conducted by Weismüller et al[56] , Kowdley et al[6] , Bugianesi E. et al[22] and Valenti et al[15] demonstrated that increased SF levels are a predictor of mortality in patients with end stage liver disease and concluded that SF levels are associated with progression of liver fibrosis in NAFLD.

# SUMMARY

1. The mean age of patients enrolled in our study was 45.99 +/- 12.17 years with the maximum number of patients in the age group of 41-50 years.
2. It is a male predominant study with 74% male patients.
3. In our study, majority of patients were obese (52%), overweight (27%). Thus, higher prevalence of NAFLD was seen in patients with higher BMI.
4. Various risk factors of NAFLD were assessed and it was concluded that majority of patients had diabetes, hypertriglyceridemia and obesity. Almost same percentage of patients were normotensive and hypertensive. Thus, it was seen that diabetes and hypertriglyceridemia had a strong association with NAFLD which was statistically significant.
5. Serum ferritin is a readily available, low-cost and non-invasive laboratory test, which is used as a predictor of hepatocellular aggression and liver fibrosis. We studied the association of hyperferritinemia with severity of fibrosis on Fibroscan. Significant relationship was seen between the serum ferritin level and liver stiffness in patients with NAFLD, and we can use serum ferritin level as a noninvasive economic option in comparison with elastography for predicting the severity of liver fibrosis among NAFLD patients.
6. Serum ferritin can be used as a screening tool in NAFLD patients to assess the severity and to plan further clinical management.
7. In our study, Serum ferritin values were defined to differentiate between high and low grades of fibrosis. SF Levels of more than 500ng/ml showed higher degree of fibrosis (F3 and F4) whereas SF levels below 500ng/ml were likely to have mild fibrosis.

## CONCLUSION

We concluded that grades of fibrosis in NAFLD was higher in patients of advancing age, presence of metabolic syndrome components i.e. obesity, diabetes mellitus and hypertriglyceridemia with a male predilection. We concluded that there is a significant relationship between serum ferritin levels and liver stiffness as seen on fibroscan in patients with NAFLD. Thus, we can use serum ferritin level as a noninvasive, easy available and an economic option for predicting the severity of liver fibrosis among NAFLD patients.

## LIMITATIONS

1. Small sample size of 100 patients and a single centre study
2. Long term follow up of patients was not done, therefore long term prognosis regarding mortality and morbidity from NAFLD could not be assessed.
3. Iron overloaded conditions were not investigated in the study.

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| SERIAL NO. | NAME | MRD NO. | AGE/SEX | BMI | DIABETES | HBA1C | HTN | HYPERTRIGLYCERDIMEA | OBESITY | ALCOHOL | SMOKING | Hb | BILIRUBIN | SGOT | SGPT | FERRITIN | VIRAL MARKERS | USG AB | FIBRROSIS SCORE | STEATOSIS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 702692 | 56/M | 22.2 | YES | 6.8 | YES | YES | NO | NO | YES | 11.8 | 1.02 | 43 | 78 | 348.6 | non-reactive | FL Grade II | 4.1 | 243 |
| 2 | J | 739479 | 48/F | 27.6 | YES | 6.7 | YES | YES | NO | NO | NO | 10.8 | 0.98 | 56 | 66 | 367.8 | non-reactive | FL Grade II | 5.6 | 266 |
| 3 | R | 977386 | 44/M | 26.2 | YES | 6.2 | YES | NO | NO | NO | NO | 13.2 | 0.78 | 30 | 66 | 238.5 | non-reactive | FL Grade I | 3.9 | 230 |
| 4 | R | 271249 | 34/M | 20.2 | NO | 5.4 | YES | NO | NO | NO | YES | 10.8 | 0.6 | 40 | 77 | 236.2 | non-reactive | FL Grade I | 4.1 | 224 |
| 5 | P | 372442 | 48/M | 36.1 | YES | 8.8 | YES | YES | YES | NO | NO | 12.6 | 1.5 | 55 | 102 | 543 | non-reactive | FL Grade III | 9.8 | 310 |
| 6 | R | 980286 | 56/M | 33.5 | YES | 8.1 | YES | YES | YES | NO | NO | 10.4 | 1.4 | 40 | 108 | 528 | non-reactive | FL Grade III | 9.3 | 313 |
| 7 | PD | 591002 | 62/F | 31.9 | YES | 7.6 | YES | YES | NO | NO | NO | 11.2 | 0.89 | 40 | 178 | 650 | non-reactive | FL Grade III | 10.2 | 274 |
| 8 | H | 595262 | 43/F | 32.2 | YES | 11.1 | YES | YES | YES | NO | NO | 13.6 | 1.3 | 70 | 170 | 686 | non-reactive | FL Grade III | 11.2 | 321 |
| 9 | R | 440012 | 48/M | 25.1 | NO | N/A | NO | YES | NO | NO | NO | 10.4 | 0.6 | 50 | 68 | 366.3 | non-reactive | FL Grade II | 6.1 | 299 |
| 10 | K | 981966 | 52/M | 31.1 | YES | 9.6 | NO | YES | YES | NO | YES | 11.9 | 0.8 | 52 | 122 | 669.8 | non-reactive | FL Grade III | 11 | 292 |
| 11 | PK | 535631 | 28y/M | 25.6 | YES | 7 | NO | NO | NO | NO | YES | 12.5 | 0.5 | 32 | 50 | 267.8 | non-reactive | FL Grade I | 4.6 | 248 |
| 12 | P | 869363 | 65y/F | 20.2 | YES | 6.2 | NO | NO | NO | NO | NO | 11.2 | 0.77 | 30 | 68 | 156.5 | non-reactive | FL Grade I | 3.2 | 203 |
| 13 | R | 983351 | 45y/F | 26.2 | NO | N/A | YES | NO | NO | NO | NO | 10.8 | 0.68 | 56 | 98 | 315.3 | non-reactive | FL Grade II | 5.5 | 281 |
| 14 | S | 982352 | 44y/F | 24.9 | YES | 6.4 | YES | NO | NO | NO | NO | 11.2 | 0.44 | 45 | 52 | 186.6 | non-reactive | FL Grade I | 3.6 | 231 |
| 15 | DS | 781956 | 26Y/M | 36.1 | YES | 10.2 | YES | NO | YES | NO | NO | 13.1 | 0.78 | 33 | 76 | 620.8 | non-reactive | FL Grade III | 9.1 | 317 |
| 16 | J | 982950 | 52y/F | 33.2 | YES | 9.8 | YES | YES | YES | NO | NO | 13.6 | 1.1 | 70 | 134 | 530.5 | non-reactive | FL Grade III | 9.5 | 305 |
| 17 | S | 503356 | 54y/M | 33.8 | YES | 10.3 | YES | YES | YES | NO | NO | 11.9 | 0.9 | 56 | 118 | 710.4 | non-reactive | FL Grade III | 11.8 | 366 |
| 18 | D | 988294 | 59Y/M | 37.1 | YES | 9.8 | YES | YES | YES | NO | NO | 12.4 | 2.1 | 66 | 120 | 600.9 | non-reactive | FL Grade III | 11.5 | 341 |
| 19 | RS | 985696 | 40Y/M | 25.2 | NO | 5.6 | NO | NO | NO | NO | YES | 13.6 | 1.7 | 50 | 90 | 210.3 | non-reactive | FL Grade II | 5.5 | 281 |
| 20 | I | 987595 | 30y/M | 27.4 | YES | 6.3 | NO | YES | NO | NO | YES | 11.2 | 0.55 | 44 | 82 | 375.6 | non-reactive | FL Grade II | 5.3 | 286 |
| 21 | R | 879069 | 60y/M | 39.2 | YES | 11.4 | NO | YES | YES | NO | NO | 10.6 | 0.89 | 42 | 176 | 538.4 | non-reactive | FL Grade III | 9.3 | 300 |
| 22 | T | 981807 | 41y/M | 33.9 | YES | 10.5 | NO | YES | YES | NO | YES | 11 | 1.34 | 78 | 148 | 580.7 | non-reactive | FL Grade III | 10.7 | 360 |
| 23 | C | 948458 | 44y/M | 38.2 | YES | 10.9 | NO | YES | YES | NO | YES | 10.8 | 1.45 | 72 | 188 | 790.7 | non-reactive | FL Grade III | 11.2 | 386 |
| 24 | D | 977496 | 60y/M | 37.1 | YES | 11.1 | YES | YES | YES | NO | NO | 11.2 | 1.23 | 66 | 86 | 645.2 | non-reactive | FL Grade III | 9.4 | 313 |
| 25 | G | 928067 | 29y/M | 33.8 | YES | 9.3 | YES | YES | YES | NO | NO | 10.7 | 1.34 | 58 | 112 | 589.6 | non-reactive | FL Grade III | 9.8 | 307 |
| 26 | J | 709057 | 31y/M | 39.1 | YES | 9.6 | YES | YES | YES | NO | NO | 11.4 | 1.67 | 54 | 167 | 710.2 | non-reactive | FL Grade III | 10.9 | 325 |
| 27 | J | 982118 | 65Y/M | 26.7 | NO | N/A | YES | YES | NO | NO | NO | 10.8 | 0.89 | 68 | 74 | 388.4 | non-reactive | FL Grade II | 5.6 | 295 |
| 28 | NS | 983707 | 50Y/M | 28.4 | NO | N/A | YES | YES | NO | NO | YES | 11.2 | 0.77 | 56 | 88 | 465.4 | non-reactive | FL Grade II | 7.7 | 287 |
| 29 | GK | 218896 | 32Y/M | 25.7 | YES | 6.1 | YES | YES | NO | NO | YES | 10.9 | 0.78 | 44 | 74 | 413.4 | non-reactive | FL Grade II | 8.2 | 281 |
| 30 | U | 467112 | 50Y/M | 33.2 | YES | 9 | YES | YES | YES | NO | NO | 11.2 | 1.22 | 68 | 122 | 789.3 | non-reactive | FL Grade III | 9.1 | 320 |
| 31 | A | 980704 | 66Y/M | 20.8 | NO | N/A | YES | YES | NO | NO | NO | 11.3 | 0.89 | 24 | 68 | 356.3 | non-reactive | FL Grade II | 6.5 | 285 |
| 32 | S | 879510 | 41Y/M | 34.8 | YES | 8.2 | NO | YES | YES | NO | YES | 11.9 | 1.34 | 44 | 123 | 613.3 | non-reactive | FL Grade III | 9.6 | 325 |
| 33 | HK | 972229 | 55Y/F | 32.2 | NO | N/A | NO | YES | YES | NO | NO | 13.1 | 1.67 | 66 | 162 | 656.4 | non-reactive | FL Grade III | 10.5 | 381 |
| 34 | C | 983875 | 30Y/M | 30.6 | NO | N/A | NO | YES | YES | NO | NO | 12.5 | 1.1 | 48 | 66 | 544.3 | non-reactive | FL Grade III | 8.9 | 296 |
| 35 | R | 751727 | 67Y/M | 27.2 | YES | 8.3 | NO | YES | NO | NO | NO | 11.4 | 1 | 67 | 87 | 523.7 | non-reactive | FL Grade III | 8.1 | 297 |
| 36 | P | 941722 | 39Y/M | 28.4 | NO | N/A | NO | YES | NO | NO | YES | 10.6 | 0.8 | 55 | 75 | 483.3 | non-reactive | FL Grade III | 9.1 | 277 |
| 37 | S | 829510 | 54Y/F | 41.1 | YES | 13.2 | YES | YES | YES | NO | NO | 12.4 | 1.56 | 89 | 167 | 718.4 | non-reactive | FL Grade III | 10.1 | 379 |
| 38 | RP | 959838 | 64Y/M | 28.9 | YES | 6.9 | YES | NO | NO | NO | NO | 11.9 | 0.8 | 57 | 73 | 222.4 | non-reactive | FL Grade II | 6.24 | 276 |
| 39 | SN | 962430 | 62Y/M | 33.9 | YES | 9.6 | YES | YES | YES | NO | YES | 12.1 | 1.2 | 62 | 101 | 639.4 | non-reactive | FL Grade III | 10.2 | 302 |
| 40 | B | 962121 | 62Y/M | 37.8 | YES | 10.2 | YES | YES | YES | NO | NO | 10.7 | 1.66 | 77 | 154 | 711.2 | non-reactive | FL Grade III | 10.8 | 381 |
| 41 | J | 939625 | 40Y/M | 36.5 | YES | 7.7 | YES | YES | YES | NO | NO | 11.2 | 0.86 | 53 | 91 | 359.7 | non-reactive | FL Grade II | 6.9 | 298 |
| 42 | J | 962343 | 59Y/M | 31.8 | NO | 5.6 | YES | YES | YES | NO | YES | 10.2 | 1.45 | 34 | 111 | 745.3 | non-reactive | FL Grade III | 11.2 | 355 |
| 43 | N | 951541 | 36Y/M | 21.2 | NO | N/A | YES | NO | NO | NO | YES | 10.3 | 0.6 | 41 | 69 | 298.3 | non-reactive | FL Grade II | 7.8 | 262 |
| 44 | J | 963908 | 54Y/M | 39.1 | YES | 9.9 | YES | YES | YES | NO | YES | 11.2 | 1.45 | 88 | 133 | 843.4 | non-reactive | FL Grade III | 12.2 | 358 |
| 45 | RG | 962135 | 45Y/M | 28.6 | YES | 6.7 | NO | NO | NO | NO | NO | 12.5 | 0.56 | 31 | 49 | 212.3 | non-reactive | FL Grade II | 4.1 | 270 |
| 46 | PD | 85883 | 59Y/M | 34.2 | YES | 9.8 | NO | YES | YES | NO | NO | 13.2 | 1.23 | 52 | 111 | 621.4 | non-reactive | FL Grade III | 9.6 | 315 |
| 47 | S | 962845 | 49Y/M | 22.5 | YES | 6.2 | NO | NO | NO | NO | NO | 10.8 | 0.3 | 23 | 45 | 123.5 | non-reactive | FL Grade I | 6.3 | 210 |
| 48 | B | 963151 | 45Y/F | 31.2 | YES | 8.4 | NO | YES | YES | NO | NO | 12.1 | 1.2 | 33 | 61 | 714.4 | non-reactive | FL Grade III | 13 | 309 |
| 49 | K | 963596 | 53Y/F | 20.4 | NO | N/A | NO | NO | NO | NO | NO | 10.1 | 0.4 | 32 | 41 | 81.3 | non-reactive | Normal | 2.13 | 146 |
| 50 | SK | 196620 | 51Y/F | 23.33 | YES | 6.4 | NO | YES | NO | NO | NO | 11.3 | 0.9 | 23 | 66 | 366.5 | non-reactive | FL Grade II | 4.3 | 295 |
| 51 | A | 964151 | 45Y/M | 35.2 | YES | 8.8 | NO | YES | YES | NO | YES | 10.7 | 1.04 | 63 | 112 | 830.5 | non-reactive | FL Grade III | 13.2 | 367 |
| 52 | SK | 488298 | 55Y/F | 26.7 | NO | N/A | NO | YES | NO | NO | NO | 12.8 | 0.78 | 35 | 76 | 398.5 | non-reactive | FL Grade II | 7.3 | 272 |
| 53 | UN | 727920 | 43Y/M | 25.7 | YES | 6.3 | YES | YES | NO | NO | NO | 12.2 | 1.03 | 44 | 74 | 376.4 | non-reactive | FL Grade II | 5.6 | 281 |
| 54 | AK | 959429 | 30Y/F | 33.4 | YES | 7.8 | NO | YES | YES | NO | NO | 10.8 | 1.66 | 75 | 116 | 642.9 | non-reactive | FL Grade III | 10.36 | 349 |

| SERIAL NO. | NAME | MRD NO. | AGE/SEX | BMI | DIABETES | HBA1C | HTN | HYPERTRIGLYCERDIMEA | OBESITY | ALCOHOL | SMOKING | Hb | BILIRUBIN | SGOT | SGPT | FERRITIN | VIRAL MARKERS | USG AB | FIBRROSIS SCORE | STEATOSIS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | MA | 958376 | 25Y/M | 37.6 | YES | 8.9 | YES | YES | YES | NO | YES | 11.4 | 1.08 | 49 | 89 | 512 | non-reactive | FL Grade III | 9.5 | 302 |
| 56 | P | 255105 | 28Y/F | 30.2 | YES | 9.5 | YES | YES | YES | NO | NO | 10.9 | 1.56 | 61 | 131 | 558.1 | non-reactive | FL Grade III | 9.2 | 303 |
| 57 | S | 951529 | 43Y/M | 21.21 | NO | N/A | NO | NO | NO | NO | NO | 11.2 | 1.03 | 22 | 52 | 188.6 | non-reactive | FL Grade II | 4.3 | 266 |
| 58 | P | 968122 | 30Y/M | 31.2 | YES | 10.2 | YES | YES | YES | NO | NO | 10.8 | 1.67 | 77 | 189 | 728.4 | non-reactive | FL Grade III | 11.6 | 356 |
| 59 | PB | 958262 | 42Y/F | 34.9 | YES | 10.7 | YES | YES | YES | NO | NO | 11.2 | 1.76 | 69 | 199 | 810.6 | non-reactive | FL Grade III | 12.7 | 372 |
| 60 | MS | 956925 | 68Y/M | 21.66 | YES | 6.3 | YES | YES | NO | NO | NO | 10.7 | 0.78 | 55 | 87 | 358.4 | non-reactive | FL Grade II | 4.9 | 283 |
| 61 | SPS | 490613 | 75Y/M | 25.9 | YES | 6.6 | NO | YES | NO | NO | NO | 11.2 | 1.16 | 62 | 103 | 489.9 | non-reactive | FL Grade II | 8.6 | 327 |
| 62 | HK | 956195 | 30Y/F | 38.2 | YES | 9.9 | NO | YES | YES | NO | NO | 10.6 | 1.55 | 86 | 198 | 340.5 | non-reactive | FL Grade III | 7.5 | 326 |
| 63 | R | 947684 | 30Y/M | 37.1 | YES | 8.5 | NO | YES | YES | NO | NO | 12.6 | 1.55 | 83 | 119 | 339.6 | non-reactive | FL Grade III | 7.9 | 380 |
| 64 | M | 950991 | 25Y/M | 32.2 | YES | 7.8 | YES | YES | YES | NO | YES | 10.8 | 1.3 | 47 | 103 | 376.7 | non-reactive | FL Grade III | 8.1 | 325 |
| 65 | AS | 217191 | 55Y/M | 25.5 | YES | 6.8 | YES | YES | NO | NO | YES | 11.2 | 0.88 | 35 | 89 | 201.1 | non-reactive | FL Grade I | 7.3 | 298 |
| 66 | GS | 951847 | 60Y/M | 26.48 | YES | 7.1 | YES | YES | NO | NO | YES | 12.2 | 0.67 | 86 | 97 | 362.2 | non-reactive | FL Grade II | 6.1 | 306 |
| 67 | MK | 938414 | 40Y/F | 34.5 | YES | 11.3 | YES | YES | YES | NO | NO | 11.8 | 1.27 | 66 | 104 | 900.6 | non-reactive | FL Grade III | 12.4 | 336 |
| 68 | KK | 951332 | 44Y/F | 25.68 | YES | 6.5 | YES | YES | NO | NO | NO | 13.2 | 1.05 | 53 | 77 | 412 | non-reactive | FL Grade II | 7.2 | 281 |
| 69 | AK | 752812 | 54Y/M | 32.1 | YES | 7.2 | NO | YES | YES | NO | YES | 10.2 | 1.58 | 66 | 83 | 358.5 | non-reactive | FL Grade II | 7.1 | 329 |
| 70 | R | 946623 | 41Y/M | 37.9 | YES | 8.8 | NO | YES | YES | NO | NO | 12.1 | 1.44 | 58 | 177 | 666.9 | non-reactive | FL Grade III | 11.1 | 341 |
| 71 | B | 931968 | 65Y/M | 22.8 | YES | 6.9 | NO | NO | NO | NO | NO | 11.4 | 0.78 | 89 | 222 | 144.5 | non-reactive | FL Grade I | 5.6 | 253 |
| 72 | R | 940408 | 50Y/M | 33.2 | YES | 8.5 | NO | YES | YES | NO | NO | 11.1 | 1.33 | 65 | 90 | 548.8 | non-reactive | FL Grade III | 9.5 | 331 |
| 73 | S | 876683 | 29Y/M | 21.6 | NO | N/A | NO | NO | NO | NO | NO | 10.2 | 0.44 | 43 | 52 | 72.2 | non-reactive | FL Grade I | 4.6 | 211 |
| 74 | MK | 855151 | 45Y/F | 20.3 | NO | N/A | YES | YES | NO | NO | NO | 10.6 | 0.76 | 44 | 93 | 178.9 | non-reactive | FL Grade II | 5.1 | 292 |
| 75 | S | 947944 | 36Y/M | 35.6 | NO | 5.8 | NO | YES | YES | NO | NO | 11.4 | 1.15 | 52 | 77 | 590.9 | non-reactive | FL Grade III | 8.9 | 308 |
| 76 | SK | 680899 | 45Y/F | 25.1 | YES | 6.2 | YES | YES | NO | NO | NO | 12.4 | 0.67 | 45 | 66 | 323.7 | non-reactive | FL Grade II | 5.8 | 294 |
| 77 | S | 790687 | 37Y/M | 33 | YES | 7 | NO | YES | YES | NO | NO | 13.1 | 1.34 | 44 | 65 | 779.9 | non-reactive | FL Grade III | 9.1 | 304 |
| 78 | A | 944326 | 76Y/M | 31.9 | YES | 8.2 | YES | YES | YES | NO | YES | 11 | 1.22 | 76 | 188 | 823.6 | non-reactive | FL Grade III | 12.4 | 336 |
| 79 | G | 956932 | 40Y/M | 36 | YES | 7.2 | NO | YES | YES | NO | YES | 12 | 1.52 | 67 | 199 | 819.4 | non-reactive | FL Grade III | 11.1 | 324 |
| 80 | SS | 987651 | 40Y/M | 32.1 | YES | 6.9 | YES | YES | YES | NO | NO | 13.2 | 1.74 | 77 | 176 | 700.7 | non-reactive | FL Grade III | 10.4 | 309 |
| 81 | RK | 110740 | 30Y/M | 35.4 | NO | 5.4 | NO | YES | YES | NO | NO | 10.9 | 1.22 | 89 | 165 | 612.2 | non-reactive | FL Grade III | 9.4 | 306 |
| 82 | Y | 200930 | 30Y/M | 22.68 | NO | N/A | NO | NO | NO | NO | NO | 10.4 | 0.55 | 16 | 40 | 78.8 | non-reactive | FL Grade I | 4.4 | 178 |
| 83 | PA | 985370 | 50Y/F | 36.2 | YES | 7.5 | NO | YES | YES | NO | NO | 12 | 1.69 | 66 | 168 | 790.4 | non-reactive | FL Grade III | 10 | 379 |
| 84 | BS | 456326 | 35Y/M | 23.44 | NO | N/A | NO | YES | NO | NO | YES | 12.8 | 0.65 | 67 | 79 | 277.7 | non-reactive | FL Grade II | 4.8 | 255 |
| 85 | HS | 362611 | 35Y/M | 21.68 | NO | N/A | NO | NO | NO | NO | YES | 13.1 | 0.47 | 44 | 64 | 133.4 | non-reactive | FL Grade I | 5.9 | 217 |
| 86 | K | 4398 | 49Y/M | 21.91 | YES | 6.4 | YES | YES | NO | NO | NO | 11 | 1.2 | 45 | 59 | 76.6 | non-reactive | FL Grade I | 4.3 | 297 |
| 87 | A | 694262 | 34Y/M | 33.1 | YES | 7.8 | NO | YES | YES | NO | YES | 11.4 | 1.1 | 102 | 267 | 667.7 | non-reactive | FL Grade III | 10.1 | 384 |
| 88 | MK | 984367 | 62Y/M | 21.66 | YES | 6.6 | YES | NO | NO | NO | NO | 10.8 | 0.68 | 68 | 88 | 200.6 | non-reactive | FL Grade II | 5 | 243 |
| 89 | B | 967730 | 47Y/M | 27.42 | NO | N/A | YES | YES | NO | NO | YES | 13 | 1.2 | 42 | 76 | 389.6 | non-reactive | FL Grade II | 6.8 | 294 |
| 90 | R | 956595 | 35Y/F | 32.2 | YES | 7.4 | NO | YES | YES | NO | YES | 12.5 | 1.35 | 88 | 106 | 648.8 | non-reactive | FL Grade III | 9.3 | 328 |
| 91 | YK | 649650 | 59Y/M | 23.1 | NO | N/A | YES | YES | NO | NO | NO | 11.4 | 0.78 | 56 | 58 | 365 | non-reactive | FL Grade II | 7.5 | 247 |
| 92 | JS | 905853 | 38Y/M | 28.12 | NO | N/A | YES | YES | NO | NO | NO | 12.5 | 0.88 | 65 | 62 | 347.7 | non-reactive | FL Grade II | 7.5 | 294 |
| 93 | SA | 986927 | 48Y/F | 26.67 | NO | N/A | YES | YES | NO | NO | NO | 11.1 | 0.56 | 38 | 58 | 411.9 | non-reactive | FL Grade II | 6.8 | 239 |
| 94 | RM | 988167 | 40Y/M | 28.99 | YES | 7.1 | YES | YES | NO | NO | YES | 12.6 | 1.21 | 40 | 65 | 348.9 | non-reactive | FL Grade II | 7.4 | 299 |
| 95 | PG | 278687 | 35Y/M | 33.4 | YES | 7.8 | NO | YES | YES | NO | YES | 13.5 | 1.44 | 66 | 210 | 778.4 | non-reactive | FL Grade III | 10.4 | 310 |
| 96 | SS | 986925 | 42Y/F | 25.9 | NO | N/A | NO | NO | NO | NO | NO | 13.8 | 0.67 | 56 | 66 | 298.1 | non-reactive | FL Grade II | 7.5 | 244 |
| 97 | K | 988424 | 60Y/F | 38.1 | YES | 8.3 | YES | YES | YES | NO | YES | 12.6 | 1.36 | 88 | 120 | 721.1 | non-reactive | FL Grade III | 10.4 | 353 |
| 98 | DS | 988622 | 34Y/M | 28.77 | NO | N/A | NO | YES | NO | NO | YES | 14.6 | 1.05 | 60 | 108 | 367.7 | non-reactive | FL Grade II | 7.6 | 303 |
| 99 | GS | 987354 | 36Y/M | 21.8 | NO | N/A | NO | YES | NO | NO | YES | 14.5 | 0.77 | 34 | 56 | 469 | non-reactive | FL Grade II | 7.5 | 260 |
| 100 | TS | 301234 | 47Y/M | 39.8 | YES | 10.2 | YES | YES | YES | NO | NO | 13.6 | 2.1 | 110 | 280 | 723.6 | non-reactive | FL Grade III | 12.5 | 400 |

Baba Farid University
of Health Sciences
Sadiq Road
Faridkot-151 203 (Pb). INDIA.
Phone : 01639-256232,256236
Tele/Fax : 01639-256235
Email – Examination@bfuhs.ac.in

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No. BFUHS/2K19p-TH/ 8291 Date 13/8/2019

The Principal,
**DMC, Ludhiana.**

**Subject:** Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.05.2019 has approved the plan of thesis of Dr. Uday Pratap Singh, a student of your college for MD General Medicine along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
To study correlation between serum ferritin levels and fibroscan among patients of non alcoholic fatty liver disease

Supervisor
Dr. Dinesh Jain
Prof.,
Dept. of Medicine,
DMCH, Ludhiana..

Co-Supervisor
Dr. Varun Mehta
Assoc. Prof.,
Dept. of Gastroenterology,
DMCH, Ludhiana.

The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,( if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

*Note :*

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

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# TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

MEDICINE

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

FEBRUARY, 2019

DR. UDAY PRATAP SINGH

DEPARTMENT OF MEDICINE
DAYANAND MEDICAL COLLEGE & HOSPITAL
LUDHIANA

# ABSTRACT OF PLAN OF THESIS

| | |
|---|---|
| Title | TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE |
| For the degree of | MD MEDICINE |
| Name of the candidate | Dr. Uday Pratap Singh |
| Supervisor | Dr. Dinesh Jain, Professor, Department of Medicine, DMCH, Ludhiana |
| Co-supervisor | Dr. Varun Mehta, Associate Professor, Department of Gastroenterology, DMCH, Ludhiana |
| Institution | Dayanand Medical College & Hospital, Ludhiana |
| University | Baba Farid University of Health Sciences, Faridkot |

**Introduction:** Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in developed as well as in developing countries. Non-alcoholic fatty liver disease represents a wide spectrum of conditions ranging from fatty liver, which in general follows a benign non-progressive clinical course, to non-alcoholic steatohepatitis (NASH), a more serious form of NAFLD that may progress to cirrhosis and end-stage liver disease.

**Aim of Study:** 1. To find a correlation between degree of fibrosis and serum ferritin levels in patients of NAFLD.

2. To define values of serum ferritin which could differentiate high and low grade of fibrosis.

**Materials & Methods:** This will be a prospective study conducted on all NAFLD patients who come to DMCH in department of Medicine & Gastroenterology for fibroscan during a period of 1 year.

**Clinical Significance**: NAFLD is the leading cause of CLD in the world. Its has association with many pre existing conditions like the metabolic syndrome, such as obesity, type 2 diabetes, hyperlipidemia and hypertension.

Liver biopsy is the gold standard for NAFLD diagnosis but being an invasive tool its role is controversial. Many non invasive methods like Transient Elastography (fibroscan) are being used instead. Serum ferritin has been found to be elevated in NAFLD & is an independent predictor of the severity and advanced fibrosis among patients.

**Keywords:** NAFLD, S.ferritin, Transient Elastography.

| (Signature) | (Signature) | (Signature) | (Signature) |
|---|---|---|---|
| Candidate | Co-supervisor | Supervisor | Principal |

## CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis entitled "**TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE**" exist in the Department of Medicine, Dayanand Medical College & Hospital, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate himself under our supervision and guidance.

**Supervisor**

**Dr. Dinesh Jain**
Professor
Department of Medicine
DMCH, Ludhiana

**Co-Supervisor**

**Dr. Varun Mehta**
Associate Professor
Department of Gastroenterology
DMCH, Ludhiana

**Head of the Department**

**Dr. Dinesh Gupta**
Professor & Head
Department of Medicine
DMCH, Ludhiana

Place : _____

Date : _____

**APPROVAL PROFORMA**
**BY**
**RESEARCH & ETHICAL COMMITTEE**
**DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA**

| | |
|---|---|
| **Name of candidate** | DR. UDAY PRATAP SINGH |
| **Department** | MEDICINE |
| **Topic of Thesis** | TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE |
| **Likely date of appearing in PG examination** | JUNE 2021 |
| **Date of enrollment** | 10th MAY 2018 |
| **Name of Head of Department** | DR. DINESH GUPTA |
| **1) Supervisor**<br>**2) Co-Supervisor** | DR. DINESH JAIN<br>DR. VARUN MEHTA |
| **Signature of Members of Research Committee with Stamp** | **Signature of Members of Ethics Committee with Stamp** |
| 1. HOD Pharmacology | 1. HOD Physiology |
| 2. HOD Surgery | 2. HOD Pathology |
| 3. HOD Pathology | 3. HOD Pharmacology |
| 4. HOD Medicine | 4. HOD SPM |
| 5. Dean Academics | 5. HOD Medicine |
| 6. Vice Principal | 6. HOD Surgery |
| | 7. HOD Obst. & Gynae. |
| | 8. Legal Expert |
| | 9. Secretary, IEC |
| | 10. Dean Academics |
| | 11. Vice Principal |
| | 12. Principal |

**Approved: Yes / No**

**Chairperson**
**Research Committee**

**Approved: Yes / No**

**Chairperson**
**Ethics Committee**

# BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

## PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

| | | |
|---|---|---|
| 1 | **NAME OF THE CANDIDATE AND ADDRESS** | DR UDAYPRATAP SINGH<br>H-283 RAMA KRISHNA VIHAR<br>29 I.PEXTN.,PATPARGANJ<br>NEW DELHI,110092 |
| 2 | **NAME OF THE INSTITUTE** | DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA |
| 3 | **COURSE OF STUDY AND SUBJECT** | MD MEDICINE |
| 4 | **DATE OF ADMISSION OF COURSE** | 10$^{th}$MAY, 2018 |
| 5 | **TITLE OF THE TOPIC** | TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE |

## 6. BRIEF RESUME OF THE INTENDED WORK

### 6.1 NEED FOR THE STUDY

NAFLD has become the leading cause of chronic liver disease and affects 30-40% of the world population. The prevalence of NAFLD has greatly increased in patients suffering from pre-existing manifestations of the metabolic syndrome, such as obesity, type 2 diabetes, hyperlipidemia and hypertension. NAFLD is prevalent in 70% of the diabetics and in 90% of the morbidly obese essentially making it the hepatic component of the metabolic syndrome.

Based on the presence or absence of hepatocyte injury, inflammation and histological appearance, NAFLD can further be classified into NAFL (non-alcoholic fatty liver) and NASH (non- alcoholic steatohepatitis). NAFL is a milder, asymptomatic and a more easily reversible condition. On the other hand, NASH is a more severe stage of the disease with inflammation and ballooning degeneration of hepatocytes and can result in chronic liver damage, scarring and fibrosis. The progression of NAFL to NASH occurs in 20-30% of the patients, but the conditions remain clinically indistinguishable. NASH leads to continuous liver fibrosis and may develop in to cirrhosis in 10-15% of the patients over a period of 10 years, in which hepatocyte loss occurs causing a functional impairment

NAFLD has been recognized as the major risk factor in the etiology of HCC in developed and developing nations.

Diagnosis of NAFLD is made by liver biopsy which is the gold standard but as an invasive method, its role in proving NAFLD diagnosis is controversial. Recently significant progressions have been made to estimate the degree of

fibrosis and one of them being transient elastography (fibroscan). Fibroscan uses ultrasound for determining the liver stiffness and degree of fibrosis

Ferrtin being an acute phase reactant is found to be raised in several systemic inflammation such as rheumatological, infectious and neoplasic diseases. Hyperferritinemia has been detected in about one-third of the patients with NAFLD and Metabolic syndromes. It directly correlates with the severity of fibrosis. Elevated serum iron and ferritin has been found in some patients of NAFLD while none of them having genetic haemochromatosis or iron accumulation in the liver. Many mechanisms are there by which iron and liver damage can be correlated. Firstly, iron accumulated in liver causes oxidative stress with ROS production and damage to membranes, proteins and DNA. Secondly ferritin being a pro-inflammatory cytokine activates hepatic stellate cells via NFKB cascade and induce fibrogenesis.

Hepcidin plays a key role in iron accumulation in NAFLD & increased levels of this peptide has been found in these patients. Hyperferrintemia has also been implicated in the development of insulin resistance by impairing the pancreatic beta cell function due to intra-parenchymal iron deposition. Insulin stimulates ferritin synthesis via inflammatory pathways and enhances hepcidin expression.

Nonalcoholic fatty liver disease (NAFLD), tightly linked to metabolic syndrome (MS), has emerged as a leading cause of chronic liver disease worldwide with a rapidly growing prevalence in the general population, ranging between 30% and 40%, and paralleling the epidemics of obesity and type 2 diabetes mellitus (T2DM) all over the world. NAFLD encompasses a clinical-pathologic spectrum of liver diseases ranging from simple steatosis to nonalcoholic

steatohepatitis (NASH), the more aggressive form of NAFLD, which can progress to cirrhosis and its associated complications.

Unfortunately, the only validated method to diagnose NASH, the potentially evolving form of NAFLD, is liver biopsy. Nonetheless, this procedure is limited by intra and inter-observer variability, sampling errors and invasiveness, thus letting impossible its feasibility in such a large number of patients with NAFLD. Several scores have been designed in the attempt to diagnose NASH and fibrosis stage without histological data, but the debate on their real utility is still ongoing. Fibroscan is emerging as a reliable tool to identify fibrosis in a non-invasive way.

Hyperferritinemia is a frequent finding in the general population, is detected in 30%–40% of the patients with MS/NAFLD, and has been suggested as a marker of severity of the disease.

fibrosis. Ferritin resulted as a good predictor of advanced liver disease, with respect to both NASH and fibrosis.

Kowdley et al.[6] demonstrated that elevated serum ferritin is an independent predictor of histologic severity and advanced fibrosis among patients with NAFLD. He found in a cohort of 628 biopsy-proven NAFLD with hyperferritinemia that ferritin, besides being significantly associated with markers of liver damage (elevated serum ALT, AST and decreased platelets) and of iron overload (iron, transferrin-iron saturation and iron stain grade), was associated with more severe histologic features of NAFLD, including steatosis, hepatocellular ballooning, increased NAFLD Activity Score (NAS) and diagnosis of NASH.

A total of 628 NASH adult subjects (age≥18 years) with biopsy proven NAFLD (defined as >5% steatosis) and a serum ferritin measurement within six months of their fibroscan were evaluated. Subjects were divided according to sex and threshold SF value of 1.5XULN (i.e., >300 ng/ml in women and >450 ng/ml in men). Higher proportion of patients with SF>1.5XULN showed increased severity of steatosis, lobular inflammation. and fibrosis compared to patients with SF≤1.5XULN (p≤0.026). Subjects with SF >1.5XULN were also more likely to have a definite diagnosis of NASH than those with SF ≤1.5XULN (70% vs. 59%, p=0.017). The proportion of subjects with advanced (stage 3 or 4) fibrosis at higher SF levels was 32% of SF>1.5XULN, 42% SF>2.5XULN and 34% SF>3.5XULN. To investigate whether various threshold levels of elevated SF were independently associated with advanced fibrosis, a multivariate stepwise logistic regression analysis including the following variables selected a priori, known to be

associated with severity of NAFLD: age at, sex, BMI, presence of diabetes and ALT.

The study showed that serum ferritin has value as a non-invasive marker in identifying patients with NAFLD who are at increased risk of more advanced disease as indicated by a higher NAS, even among patients without hepatic iron deposition. It was found that SF>1.5XULN is independently associated with increased risk of advanced fibrosis in NAFLD patients. Results indicated that use of a SF value >1.5XULN can be of diagnostic value in patients with NAFLD.

Zelber-Sagi et al.[7] showed that NAFLD was responsible for the association between serum ferritin and the metabolic syndrome and most of its components suggesting that the relation between serum ferritin and most of metabolic syndrome features might be mediated by the presence of NAFLD at population-based level.Among the metabolic factors, insulin appeared to be the strongest predictor of increased serum ferritin levels, but the association between serum ferritin and insulin was much more evident in the NAFLD group.

Angulo et al.[8] retrospectively analyzed in 1404 NAFLD patients the accuracy of serum ferritin in determining the presence and severity of liver fibrosis, and whether combining non-invasive fibrosis scoring systems with serum ferritin analysis could increase the accuracy of those tests

Yoneda et al.[9], analyzed 1201 biopsy-proven NAFLD patients previously enrolled into the Japan Study Group of NAFLD and belonging to a large Japanese cohort database of NAFLD patients. Serum ferritin levels and

hepatic histology were compared, the authors showed that ferritin increased with increasing histological grade of steatosis, lobular inflammation.

Bugianesi et al.[10] conducted a study and demonstrated that increased ferritin levels are markers of severe liver damage, but not of iron overload .The iron burden and HFE mutations do not contribute significantly to hepatic fibrosis in the majority of patients with NAFLD was also proven.

Vecchi *et al.* [11],conducted a study and demonstrated the relationship between glucose and iron metabolism and showed a new regulatory pathway in iron homeostasis which was driven by gluconeogenic stimuli with major actors being hepcdin and PPARGC1A, a transcriptional coactivator of genes involved in gluconeogenesis.

Dongiovanni *et al.* [12], conducted a study in which an iron-enriched diet led to the development of IR,due to the secretion of adipokines by the visceral adipose tissue consequent to iron accumulation

Kim C.W 13 et al conducted a study Serum ferritin levels predict incident non-alcoholic fatty liver disease in healthy Korean men and concluded that increased ferritin levels plays a role in development of DM.

Beckry et al. [14] showed the ectopic expression of hepcidin in white adipose tissue of obese individuals and leptin, which usually increased in obese subjects, was in turn was able to enhance hepcidin mRNA *in vitro*.

Valenti et al [15] conducted a study showing that that iron depletion by venesection, in patients with moderate iron overload associated with NAFLD, determined a decrease of both IR and transaminases, as well as of ferritin levels.

Dongiovanni P [13] showed that HFE mutations responsible for hereditary hemochromatosis resulted non significantly associated either to liver siderosis or hyperferritinemia and also liver damage did not result as being influenced by the presence of these mutations.

Facchini et al [16] demonstrated that NAFLD patients, with or without increased ferritin levels, that iron removal in patients with clinical evidence of nonalcoholic fatty liver disease was able to improve insulin sensitivity in the short term

Alam et al. [17] demonstrated that obesity led to hyperferritinemia irrespective of actual body iron story, advocating a state of subclinical inflammation responsible for high levels of ferritin and increased BMI leads to hyperferrintemia.

Iwasaki T [18] conducted a study showing an association between serum ferritin, visceral fat and subcutaneous adiposity and thereby making serum ferritin concentration a useful indicator of systemic fat content and degree of IR

Green et al [19] demonstrated that isolated adipocytes exposed to iron become insulin-resistant decreasing insulin mediated glucose transport and fostering lipolisis.

## 6.3 AIMS AND OBJECTIVES OF THE STUDY

1. To find a correlation between degree of fibrosis and serum ferritin levels in patients of NAFLD.
2. To define values of serum ferritin which could differentiate high and low grade of fibrosis.

## 7. MATERIALS AND METHODS

All NAFLD patients who come in Medicine, Gastroenterology department of DMCH for fibroscan during a period of 1 year will be included in the study.

### 7.1 Source of Data

Patients of NAFLD who come to medicine/gastroenterology department for fibroscan will be included in this study.

### 7.2 METHOD OF COLLECTION OF DATA

Patients of NAFLD who come to DMCH for fibroscan will be enrolled in the study.

**INCLUSION CRITERIA** - Patients of NAFLD who come to medicine and gastroenterology department for fibroscan will be included in this study.

**EXCLUSION CRITERIA –**

- Autoimmune hepatitis
- Post viral hepatitis
- Anemia (HB<10)
- Wilsons disease
- Haemochromatosis
- Consumption of hepatotoxic drugs
- Decompensated cirrhosis
- Ascites

**DURATION OF STUDY** – 1 year

**METHODOLOGY –**

- Patients will be evaluated for metabolic syndromes such as obesity, hyperlipidemia and uncontrolled blood sugar levels.

- Levels of serum ferritin will be done and measured and a liver elastography performed to know the degree of fibrosis.
- Degree of fibrosis will be based on kpa values.
- Kpa>8.9 will be f3 and kpa>11.8 wil be f4 which will be the markers of high grade of fibrosis and f1&f2 will be markers of low grade fibrosis
- Liver elastography will be done by echo sens fibro scan.

**OUTCOME**

To correlate degree of fibrosis and serum ferritin levels in NAFLD patients.

**STATISTICAL ANALYSIS**

Data were described in terms of range; mean±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups was done using ANOVA. For comparing categorical data, Chi square ($\chi 2$) test was performed and exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

**7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.**

Study requires investigations like Haemogram, LFTS, viral markers, serum ferritin, USG abdomen, fibroscan.

Other investigations will be done if required –ANA, cerruloplasmin, KF rings examination.

**7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?**

Attached along with

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## PROFORMA

**SERIAL NO.**

**NAME-** **AGE/SEX-**

**MRD NO-** **WEIGHT-**

**HEIGHT-** **BMI-**

**PAST HISTORY-**

DIABETES MELLITUS

HYPERTENSION

OBESITY

HYPERTRIGLYCERIDEMIA

**PERSONAL HISTORY –**

ALCOHOL INTAKE YES/NO

SMOKING YES/NO

VEGETARIAN/NON VEGETARIAN

ANY H/O HEPTOTOXIC DRUGS

H/O TPN

**LAB INVESTIGATIONS**

HAEMOGLOBIN

BILIRUBIN

SGOT/SGPT

S.FERRITIN

VIRAL MARKERS

USG ABDOMEN

FIBROSCAN

ANA (WHEN REQUIRED)

CERRULOPLSMIN (WHEN REQUIRED)

KF RINGS (WHEN REQUIRED)

**SIGNATURE OF CANDIDATE** **SIGNATURE OF SUPERVISOR**

## CERTIFICATE

We hereby certify that the informed patient consent form and its translation and back translation to Hindi and Punjabi are accurate to the best of our knowledge and satisfaction.

**Supervisor**

**Dr. Dinesh Jain**
Professor
Department of Medicine
DMCH, Ludhiana

**Co-Supervisor**

**Dr. Varun Mehta**
Associate Professor
Department of Gastroenterology
DMCH, Ludhiana

**Dr. Uday Pratap Singh**
P.G. Resident
Dept. of Medicine
DMC & Hospital, Ludhiana

# मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

# ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

**"TO STUDY CORRELATION BETWEEN SERUM FERRITIN LEVELS AND FIBROSCAN AMONG PATIENTS OF NON ALCOHOLIC FATTY LIVER DISEASE"** ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____
ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ

_____
ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____

ਮਿਤੀ_____

ਪਤਾ _____

ਪਤਾ _____

_____
ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____
ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ